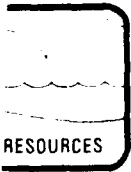


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# BIOASSESSMENT METHODOLOGIES FOR THE REGULATORY TESTING OF FRESHWATER DREDGED MATERIAL

Proceedings of a Workshop

Compiled by

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Environmental Laboratory

DEPARTMENT OF THE ARMY  
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Prepared for US Army Engineer District, St. Paul  
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## PREFACE

This report summarizes the proceedings of a workshop held in Milwaukee, Wis., for the US Army Engineer District, St. Paul, by the Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The workshop was held in response to a request from the Wisconsin Department of Natural Resources (DNR) to the St. Paul District for planning assistance under Section 22 of the Water Resources Development Act of 1974 (Public Law 93-251). The DNR was interested in identifying appropriate bioassessment testing methodologies for the regulatory testing of freshwater sediments scheduled for dredging and open-water disposal.

To identify the appropriate methodologies, the EL, in consultation with the St. Paul District and the DNR, carefully selected highly regarded individuals from private industry, the Federal government, and institutions of higher learning. The roster of workshop participants was composed of both technically oriented individuals who develop and conduct bioassessment tests as well as persons who must use the results of such tests in a regulatory decisionmaking context.

Financial support for travel and preparation of the proceedings was provided by the St. Paul District's Section 22 Office to the EL through an Intra-Army Order for Reimbursible Services. The DNR contributed generous financial support by sponsoring the travel expenses of eight non-Corps technical workshop participants.

The authors gratefully acknowledge the coordinating efforts of Mr. Stan Kummer, project manager for this work effort and Section 22 coordinator for the St. Paul District. The authors also appreciate the cooperative and logistical assistance provided by Mr. Rahim Oghalai, Dr. John Sullivan, and Mr. Scott Hausman of the DNR. Ms. Dorothy Booth of the Environmental Information Analysis Center, EL, and Ms. Jamie Leach, Publications and Graphic Arts Division, WES, are commended for providing outstanding editorial services in the publication of this proceedings.

The workshop proceedings were written by Dr. Thomas M. Dillon and Ms. Alfreda B. Gibson of the Ecosystem Research and Simulation Division (ERSD), EL. The 3-day workshop was chaired by Dr. Dillon. This project was conducted under the general supervision of Dr. Richard K. Peddicord, Team

Leader, Biological Evaluation and Criteria Team, and Dr. Charles R. Lee, Group Leader, Contaminant Mobility and Regulatory Criteria Group. The Chief of ERSD was Mr. Donald L. Robey and Chief of EL was Dr. John Harrison.

Director of WES was COL Allen F. Grum, USA. Technical Director was Dr. Robert W. Whalin.

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AGENDA

Workshop on Sediment Bioassessment Techniques  
Milwaukee, Wisconsin, 16-18 April 1985

Tuesday, 16 April 1985

- 8:15 Coffee
- 8:30 Welcoming Remarks
- 8:45 Introduction to Workshop
- 9:00 Summary of Preworkshop Inputs from Participants
- 11:30 Lunch
- 1:00 Acute Toxicity Tests
- 4:30 Break for Evening

Wednesday, 17 April 1985

- 8:15 Coffee
- 8:30 Chronic Toxicity Tests
- 12:00 Lunch
- 1:00 Other Bioassessment Techniques
- 4:30 Break for Evening

Thursday, 18 April 1985

- 8:15 Coffee
- 8:30 Bioaccumulation
- 12:00 Lunch
- 1:00 Workshop Consensus of Significant Findings
- 4:00 Critique of Workshop
- 4:30 End of Workshop

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BIOASSESSMENT METHODOLOGIES FOR THE REGULATORY TESTING  
OF FRESHWATER DREDGED MATERIAL

Proceedings of a Workshop

PART I: BACKGROUND

Initial Planning

1. In September 1984, the State of Wisconsin Department of Natural Resources (DNR) formally requested planning assistance from the US Army Engineer District, St. Paul (hereafter referred to as the District), under Section 22 of the Water Resources Development Act of 1974 (Public Law (PL) 93-251). The DNR requested assistance in developing management alternatives for contaminated sediments as described in their 3-year scope of work. See Appendix A for supporting documentation and communications pertinent to this introductory section.

2. Briefly, the scope of work described a three-phased study in which (a) bioassessment methodologies would be identified for the evaluation of sediments prior to dredging and open-water disposal in freshwater environments, (b) selected methodologies would be evaluated for their working utility and regulatory applicability, and (c) the methodologies would be exhaustively tested using a wide variety of sediment types.

3. In early October 1984, the District requested that a representative from the US Army Engineer Waterways Experiment Station (WES) provide the District with any necessary technical support in an initial planning meeting with the DNR. On 10 October 1984, such a meeting was held at the DNR headquarters in Madison, Wis. While the original scope of assistance proposed by the DNR called for a literature review to identify the bioassessment methods, the WES representative, Dr. Tom Dillon, suggested that a workshop of selected technical experts would perhaps be a more appropriate way to achieve the desired goal. Dr. Dillon indicated that he would be willing to develop a separate scope of work describing how the WES would conduct such a workshop. He also noted that he would work closely with the DNR personnel, but that his primary mission was to provide technical support to the District and to assist the District in whatever manner necessary. The District representative, Mr. Stan

Kummer, indicated his desire for WES to be involved and to work closely with him and the DNR staff. In the ensuing months, a scope of work describing the conduct of a workshop was negotiated between WES and the District with input from the DNR. (See cover letter Dillon to Kowalski, 12 March 85, Appendix A.)

#### Workshop Development

4. As an initial step in developing the workshop, Dr. Dillon requested that the DNR provide a written historical perspective of dredging in Wisconsin, which he could in turn send to prospective workshop participants. He also requested that DNR suggest the names of technical experts they would like to attend the workshop. The requested information was sent to the WES on 13 December 1984.

5. In January 1985, each prospective participant was requested to submit a list of bioassessment methodologies felt to be most appropriate for the regulatory evaluation of sediments. These inputs helped to form the basis for the workshop agenda. All preworkshop inputs from the participants were received in March 1985 and a final agenda was developed in early April 1985.

#### Conduct of the Workshop

6. The 3-day workshop was held at the Red Carpet Inn in Milwaukee, Wis., 16-18 April 1985. Certain participants were contacted prior to the workshop and requested to be prepared to give a short introduction on particular subjects. The general format of the workshop was to initiate each session with these short introductions after which the advantages and disadvantages of the subject item would be openly debated and discussed. The participants were notified prior to and at the workshop that they would be asked to prioritize the bioassessment methodologies and justify the ranking they selected.

7. Following is a detailed summary of the proceedings of the 3-day workshop containing conclusions and recommendations made by the participants.

## PART II: PROCEEDINGS OF THE WORKSHOP

### Introduction to the Workshop

8. Attendees were welcomed by Dr. Tom Dillon, the workshop moderator, who asked Dr. John Sullivan to give the Wisconsin DNR perspective on the need for this workshop. Dr. Sullivan reviewed DNR's proposed 3-year plan to identify, test, and implement bioassessment methodologies for the regulatory testing of sediments intended for open-water disposal in freshwater environments. He indicated that previous evaluations relying solely on bulk chemistry data had proven inadequate for their needs. He charged the attendees to identify one or more scientifically defensible bioassessment methodologies that could be conducted by a wide range of testing laboratories and used in a regulatory testing program.

9. Mr. Scott Hausman of DNR provided a historical perspective. Since 1971, the laws of the State of Wisconsin have prohibited the disposal of dredged material in Wisconsin's waters and riverine floodplains. Dredged material has been placed upland or nearshore in diked confined disposal facilities (CDF). In the past, the CDFs were planned and built entirely with Corps funds. In the current political climate, the Federal government requires State and local governments to fund all or part of many projects previously supported entirely by the Federal government. Mr. Hausman felt that in the near future the State would have to reconsider its total ban on open-water disposal for economic reasons. In addition, he pointed out that the environmental justification for instituting the ban on open-water disposal in the first place is being reevaluated. Consequently, the DNR would like to identify potentially useful bioassessment methodologies, so that when and if open-water disposal becomes a viable alternative in Wisconsin, the initial steps in developing regulatory testing will have already been taken.

10. Mr. Stan Kummer gave the St. Paul District's perspective and expressed his and the District Engineer's willingness to cooperate with the DNR in this workshop and in the 3-year plan. He also expressed his thanks to WES for organizing the workshop.

11. Dr. Dillon asked each of the workshop participants to introduce themselves and describe their area of expertise and years of experience with dredging and sediment testing. He also asked that they classify themselves

as a "doer" (one who develops and conducts bioassessment methodologies) or as a "user" (one who utilizes the results of the methods in a regulatory context).

12. This introductory exercise was conducted for several reasons. First, it served to let each person become acquainted with his coparticipants and their areas of expertise. Second, it indicated that there was a good balance of regulatory (five) and bench-type (nine) scientists, some of whom classified themselves as both a doer and a user (Table 1). This balance of personnel was also reflected in the participants' affiliations (six government, three industry, and two academia). Thirdly, it demonstrated that approximately 80 years of collective experience was represented by the group. This latter fact is important because it indicated that the workshop could call upon a tremendous amount of hands-on experience that would not have been available in a literature review.

#### Preworkshop Input

13. Dr. Dillon distributed a summary of the types of information received from the participants prior to the workshop (Table 2). He expressed his thanks to the participants for the clear and thorough input they had provided (Appendix B). Dr. Dillon indicated that he used this information to help formulate the final workshop agenda, which he briefly reviewed. He stated that the bioassessment methods which would be discussed during the course of the workshop were, in most instances, taken from larger testing scenarios that considered items other than bioassessment methods. He briefly discussed some of those items prior to moving on to the biological test methods.

#### Tiered (hierarchical) testing

14. Input from several participants referred to a tiered (hierarchical) testing approach. Dr. Dillon indicated that this approach was also evident in other inputs he had received, although not specifically mentioned by name. He felt that the concept of tiered testing would provide an appropriate framework for the ensuing workshop discussions and asked Dr. John Scott to briefly describe the concept.

15. Dr. John Scott described a testing protocol referred to as Hazard Assessment (HA) that incorporates the tiered testing format (Cairns, Dickson,

Table 1  
Composition of Workshop Participants

<u>Participant</u>	<u>Affiliation*</u>	<u>Work Emphasis**</u>	<u>Experience, yr†</u>
Adams	Industry	Doer	6
Alden	Academia	Doer	7
Bajek	Gov't-CE	User	5
Chapman	Gov't-EPA	Doer/User	2
Dillon	Gov't-CE	Doer/User	7
Krauser	Gov't-CE	User	5
Mac	Gov't-FWS	Doer	5
O'Connor	Academia	Doer	15
Rubinstein	Gov't-EPA	Doer/User	9
Scott	Industry	Doer	10
Ward	Industry	Doer	10
<hr/>			
11 Participants	6 Gov't	6 Doers	81 years
	3 Industry	3 Doers/Users	
	2 Academia	2 Users	

\* CE = US Army Corps of Engineers, EPA = US Environmental Protection Agency, FWS = US Fish and Wildlife Service.

\*\* Doer - one who develops and conducts bioassessment methodologies.  
 User - one who utilizes the results of the methods in a regulatory context.

† Years of experience with sediment testing and/or dredging.

Table 2  
Generalized Summary of Preworkshop Inputs from Technical Participants

Participant*	Chronic Sublethal and Other Bio-Assessment Tests**												
	Initial Predredging Assessments	Sediment Sampling	Bulk Sediment Analysis	Acute Lethality Tests	Reproductive Impairment	Ames test	Partial life cycle, histopathology, Ames test	Microcosms, AEC, bioenergetics, SCE, histopathology	Partial life cycles, bioenergetics, SCE, histopathology, AEC	Partial life cycles	Trophic Transfer	Monitoring Disposal Sites	Tiered Testing Approach
1	✓	✓	✓	✓							✓		
2	✓	✓	✓	✓							✓		
3		✓	✓	✓							✓		
4	✓	✓	✓	✓	Reproductive impairment						✓		
5			✓	✓	Ames test						✓		✓
6				✓	Partial life cycle, histopathology, Ames test						✓		✓
7				✓	Microcosms, AEC, bioenergetics, SCE, histopathology						✓		✓
8				✓	Partial life cycles, bioenergetics, SCE, histopathology, AEC						✓		✓
9			✓	✓	Partial life cycles						✓		✓
10											✓		✓

\* Numbering of participants is random and does not reflect any particular order (e.g., alphabetical).  
 \*\* AEC = adenylate energy charge; SCE = sister chromatid exchange.

and Maki 1978). HA consists of two major blocks of information, effects assessment and exposure assessment (Figure 1). Within each block is a series of tiers progressing from the simple to the more sophisticated tests. As one moves to the more sophisticated assessments, more time and effort are generally required, but the confidence in the eventual decision rendered from such data is increased. As one moves up in tiers, the level of biological organization in effects assessments generally increases in complexity from organismic to populations and communities. For example, an early tier test may be a 96-hr acute lethality test while the potential impacts on populations and communities may be considered in a much later tier.

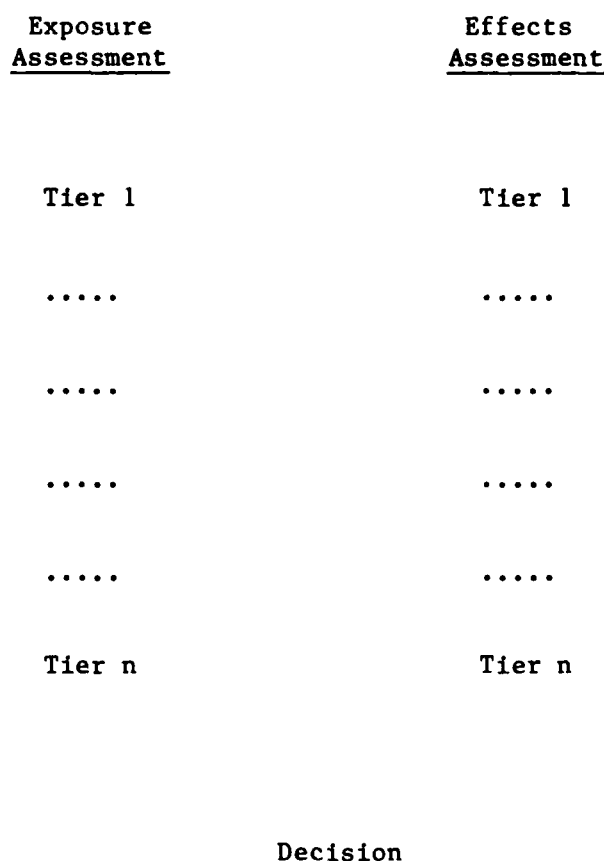


Figure 1. Schematic representation of a HA decisionmaking framework

16. There appeared to be an early favorable consensus in the group regarding the tiered testing approach for bioassessment methodologies. Several participants noted that such an approach could potentially allow time and

resources to be apportioned more judiciously and would therefore reduce the amount of unnecessary testing.

17. Dr. Dillon noted that, in his experience with tiered testing, the most difficult area was in quantifying the decision to move from one tier to another. Mr. Norm Rubinstein indicated that HA and the tiered testing approach represented a sound scientific rationale for decisionmaking. Its utilization would probably reduce the risk of litigation and, if litigated, would increase the chance of the decisionmaker winning in court.

#### Bulk sediment chemical analysis

18. Another item in the preworkshop inputs but not considered a bio-assessment methodology was bulk chemical analysis of sediments. Dr. Dillon indicated he wanted to put this issue before the group since there has been such a heavy reliance on bulk chemistry data in evaluating sediments in Wisconsin and the Great Lakes area as well as throughout the United States. The group was somewhat divided on this issue. Several participants seemed disinclined to routinely conduct a bulk analysis, citing the problem of interpreting the results. Specifically, a bulk chemistry analysis does not indicate what proportion of the total concentration of each contaminant is available for uptake into biota. Several noted that bioavailability may vary considerably from sediment to sediment and among different contaminants.

19. Other participants, agreeing with these shortcomings, argued in favor of obtaining this type of information. They said that this type of data is useful at least in the initial assessments phase since it can indicate presence or absence of certain contaminants. Dr. Joe O'Connor noted that there are simple predictive calculations currently under development by various groups throughout the United States that can be made with bulk chemistry data. These procedures generate the thermodynamically defined maximum bioaccumulation potential for neutral organic contaminants such as polychlorinated biphenyls (PCBs) (see section entitled "Bioaccumulation"). He also very much liked the idea of having at least a qualitative listing of sediment contaminants. Mr. Hausman remarked that the problem with the qualitative list is that sometimes contaminants of concern may be in the sediment but are not identified in the bulk chemistry data because the compound was either unknown at the time of the analysis or the particular analytical group did not specifically look for it. The discovery of kepone in the James River, Va., was cited as an example of this potential dilemma.



20. Dr. Sullivan said the DNR was moving away from sole reliance on bulk chemistry data and towards biological testing for reasons just described by Mr. Hausman and because of the difficulty in interpreting what the contaminant concentrations mean to organisms. There seemed to be a consensus in the group that, historically, too much emphasis has been placed on bulk chemistry data alone, and the results were often interpreted in a technically unsound manner. However, they did agree that it was useful information to have during initial sediment evaluations. Dr. O'Connor urged that, in any bulk analysis, organic carbon, grain size, and moisture content be determined in addition to contaminant concentrations so that the thermodynamically defined maximum bioaccumulation potential could be calculated during the initial assessments.

#### Bioassessment Methods

##### Acute lethality tests

21. Dr. Tim Ward led off this session by describing the acute lethality tests commonly used in the regulatory testing of dredged material scheduled for open-water ocean disposal. His presentation stimulated the discussion of several items.

22. Affecting all these discussion items was a central question: Should acute lethality tests simulate conditions at the disposal site or should there be an attempt to standardize the tests? Most participants, especially those calling themselves users, said they favored more standardized tests, even at the expense of simulating disposal site conditions. The doers said that simulating environmentally realistic conditions could be accomplished with varying degrees of sophistication. Dr. Dillon said that the tests used in the ocean dumping regulatory program were not intended to closely simulate environmentally realistic conditions but rather to assess the potential for impact. Dr. Scott pointed out the HA approach offered various degrees of environmental simulation and corresponding biological effects assessments depending on the required level of sophistication. The consensus of the group was a hybrid of these two perspectives. That is, standardizing the tests should receive highest priority, but there should be some attempt to use environmentally realistic exposure conditions whenever possible.

23. Phase testing. Dr. Ward indicated that, historically, three phases of sediment have been tested in the ocean dumping program: an elutriate or

liquid phase, a suspended particulate phase, and a solid or bedded sediment phase. He indicated that he has all but eliminated liquid phase testing in his laboratory because it was rarely acutely toxic and, after consideration of initial mixing, contained low to nondetectable levels of contaminants. There was a strong consensus among the group that liquid phase testing could be eliminated in the majority of cases. Representatives from New York District and New England Division said they no longer required liquid phase testing in their regulatory program. Dr. O'Connor said that for certain specialized tests (see section entitled "Other Bioassessment Techniques"), a liquid phase would be necessary since a solvent extract is employed.

24. There was no clear consensus concerning the value of suspended phase (SP) testing. Some participants said they rarely observed acute lethality, while others (Dr. Ray Alden, for example) relied heavily on SP testing as an initial screening tool. Dr. Scott expressed concern with eliminating all SP testing since he had observed biological effects in suspension-feeding organisms at very low SP concentrations. Dr. Dillon indicated that work at WES demonstrated that the rate and degree of bioaccumulation of neutral organic contaminants were highly dependent on the SP concentration in the exposure zone.

25. There was a clear consensus in favor of solid phase testing. This was not too surprising since field information gathered over the years has shown that, if an effect due to open-water disposal is observed, it is usually associated with the benthic environment. In addition, most participants said that, if acute lethality was seen in laboratory tests, it was usually in the solid phase. In addition, assessing the potential for bioaccumulation in many regulatory programs is usually carried out at the end of the solid phase test by analyzing the tissues of surviving organisms.

26. Mr. Rubinstein urged the participants to consider the whole sediment during solid phase testing in particular and during all regulatory tests in general. As a result of animal activity, sampling activity, or the hydraulics of the laboratory test system, a SP will be present that may be large or small and may be quite variable in space and time. Considering the reported effects of the SP on both biological effects and bioaccumulation, not controlling this factor could result in variable nonreproducible test results. Treating the material as a whole sediment would most likely also lead to test systems that more closely simulate field conditions.

27. The discussion that followed on ways to control the SP made it clear that the methodologies range from the very simple to the very sophisticated. Although there appeared to be agreement on the importance of the SP and, considering the whole sediment during testing, the group could not agree on a single method for controlling SP during solid phase testing.

28. Test organisms. One consensus reached during this session of the workshop was that a matrix of organisms should be used in any testing program and complete reliance on a single species was technically unsound. The organisms selected for testing should represent those inhabiting the near-bottom and the in-sediment environments. For near-bottom testing, a fish, a daphnid, and/or a mysid were suggested. For in-sediment tests, amphipods and larval mayflies and chironomids were suggested. Dr. Scott revealed that amphipods on the east (*Ampelisca* sp.) and west (*Rhepoxynius* sp.) coasts have been shown to be extremely sensitive to contaminated sediments. He pointed out that there was a corresponding freshwater species (*Pontoporeia* sp.) that, from the limited information available, also appeared to be very sensitive.

29. As mentioned previously, solid phase tests are often used to assess the potential for bioaccumulation. Consequently, organisms selected for this purpose should have sufficient biomass so that tissue residues can be accurately determined. Earthworms and bivalves were suggested as potentially useful organisms to assess bioaccumulation potential because of their size. Drs. Bill Adams and Gary Chapman suggested larval mayflies and chironomids because they are both sensitive species, they are large enough for chemical analysis, and they can be cultured in the laboratory (BatacCatalon and White 1982, Nebeker et al. 1984).

30. Another consensus reached by the participants was that surrogate species should be used exclusively or at least in conjunction with organisms collected at the disposal site. Surrogate species are organisms that have similar ecological requirements and are phylogenetically similar to those inhabiting the disposal area. They may be collected from relatively uncontaminated reference areas or from laboratory cultures. Dr. Michael Mac argued convincingly against the use of organisms collected at or near the disposal site since they may have developed an enhanced resistance to contaminant perturbations. The suggestion by the workshop participants to use surrogate species appeared to be largely driven by the desire to move towards standardized testing procedures. Since surrogate species are normally kept in culture

by one or more facilities, several commonly used species are likely to be available for testing throughout the year.

31. Standard reference toxicants. This discussion item emerged in reference to quality-control measures. Standard reference toxicants are compounds used to assess the sensitivity of animals by measuring their survival at various levels of exposure. If the sensitivity (i.e., survival) of groups of organisms tested successively over time is unchanged, then differences observed in regulatory tests conducted concurrently may be attributed solely to the imposed treatment. The results of standard reference toxicant bioassays, conducted simultaneously with sediment bioassays, are used to ensure that results obtained in the sediment tests are not due to a change (either increase or decrease) in the organism's sensitivity. Results of standard reference toxicant tests can also be used to make interspecific comparisons in species sensitivity.

32. Mr. Rich Krauser said that the New York District requires their permit applicants to conduct bioassays with the standard reference toxicant dodecyl sodium sulfate, which is also known as sodium lauryl sulfate. He cautioned that there are two different grades of this compound with the purer and more expensive grade being significantly more toxic than the less pure, cheaper formulation. Dr. Dillon pointed out that the American Society for Testing and Materials had published recommendations concerning the use of standard reference toxicants (Lee 1980).

33. The group agreed that there was no technical reason not to use standard reference toxicant bioassays, especially in a regulatory testing program, and that it was a laudable and worthwhile effort. It was their general impression, however, that its use was not widespread.

34. Control and reference treatments. There was considerable discussion among the workshop participants on what constitutes control and reference treatments in sediment bioassays. From the discussion that transpired, it appeared that the lack of a clear definition was not limited to this group. In classical experimental design, the control treatment controls for all variables except the treatment variable. Historically, the control in aquatic toxicology has consisted solely of clean water. However, in tests with sediments, the bioassay organism often requires sediment as a substrate and/or food source. This treatment therefore is often referred to as either the control or reference sediment treatment. Characteristics of sediments other than

contaminant concentration (e.g., grain size, organic carbon, moisture content, and hydrogen sulfide) can and do affect the organism's response in sediments. Consequently, many tests contain a reference treatment that is similar to the test sediment in all respects except for contaminant content. Clarification of the nomenclature is further hindered by the fact that many tests include an experimental treatment containing sediment collected from what is referred to as a "reference area." This reference sediment often contains measurable amounts of contaminants, but, from a regulatory standpoint, it is the reference sediment with which the test sediment results are compared.

35. There was no clear consensus on definitions of control and reference treatments. It is recommended therefore that these terms be fully explained on a case-by-case basis.

36. Site water and the flow-through alternative. These two items were discussed at the same time since they are interdependent. There was some question as to whether site water (i.e., water from the proposed disposal area) should be used during the biological tests. The primary advantage is that any possible effect due to the site water would theoretically exert its influence during the tests. The primary disadvantages are logistical and economical. These disadvantages are compounded if the tests are not static but rather are flow-through. Dr. Mac indicated that waters of the Great Lakes tended to be more similar than dissimilar. He seemed to think that a standardized list of representative water characteristics could be generated to identify waters acceptable for use in bioassays. This recommendation coincides with the more general consensus of the group to encourage more standardized tests.

37. The flow-through versus static question was discussed at length with no consensus. Mr. Rubinstein made the point that the requirements of the test organism must be met first before any experimental variable is imposed. If the organism requires flow-through conditions, then the test must be conducted using flowing water. If not, then a static exposure may be utilized. Again, if the flow-through alternative is required, then the use of site water would, in all likelihood, be economically prohibitive.

38. Temperature. The group could not recommend a specific temperature to conduct acute lethality tests, although identifying a recommended range may be possible with a minimum amount of research. Dr. Mac pointed out that disposal in the Great Lakes occurs only from May through September due to ice

formation and inclement weather. He cautioned that, when reviewing field temperature data, it should be kept in mind that the Great Lakes experience extreme stratification, and that true bottom temperatures should be considered for evaluating acute toxicity at a disposal site. Dr. Dillon suggested that if a relatively narrow range of bottom temperatures could be identified, then a single representative temperature could possibly be selected based on sound technical data. This may require some temperature acclimation of the test organism prior to testing but should not be a substantial obstacle, given a sufficiently narrow temperature range.

#### Chronic toxicity tests

39. Microcosms. Dr. Ray Alden initiated this discussion by describing the laboratory microcosm approach he has developed. He has observed in the laboratory significant changes in established benthic and zooplankton communities as a result of simulated disposal of dredged material. However, the effects were less dramatic than one would expect from the results of parallel static bioassays. He has also observed that the accumulation of polycyclic aromatic hydrocarbons by bivalves was slightly greater in the microcosms than in static bioassays. Although he could not explain this observation with any certainty, he did feel it was due to the more natural conditions existing in the microcosm (e.g., currents, food supply, interspecific interactions) relative to the static bioassays. Although the costs to develop and run the microcosm can be quite substantial, a very large data set can be generated regarding the toxicity of sediments to major taxa indigenous to the disposal area. He estimated these costs to approximate those required to conduct multiple species bioassays.

40. Although Dr. Alden has not often observed toxicity or differences in community structure, he did report that, on a number of occasions, he observed benthic fauna leaving the sediment and entering the water column. The workshop participants were all very interested in this observation. Dr. Dillon pointed out that, from an ecological perspective, such behavior could be interpreted as a lethal response since the meiofauna in the field would probably experience intense predation pressure. Mr. Rubinstein suggested this avoidance response behavior may be useful in showing which species or groups of species are particularly sensitive to contaminated sediments. It was noted that microcosm design is not standardized and varies considerably among laboratories. Also, seasonal effects generally prevent

microcosm tests from being considered reproducible. However, Dr. Alden noted that although the species comprising various communities may vary seasonally and from year to year, the very toxic sediments do consistently affect the more sensitive species in the community.

41. Life cycle test. Dr. Adams initiated this discussion by describing the partial and full life cycle tests he conducts with the freshwater midge *Chironomus tentans*. He indicated that the full life cycle can be completed in 30 to 40 days and that the fourth instar midge is large enough (up to 30 mm, 25 mg wet weight) to assess bioaccumulation potential. In assessing sediment toxicity, he carries out life cycle studies on both *C. tentans* and the water flea *Daphnia magna*. He also indicated that with his system, sediment/water partition coefficients can be calculated if chemical analysis of sediment and water samples is conducted. The group responded quite favorably to Dr. Adam's description of tests for evaluating the acute toxicity, bioaccumulation potential, and chronic life cycle effects of sediments.

42. Life cycle tests with other freshwater organisms living in or on the sediment were discussed. Species mentioned included the amphipods *Gammarus*, *Hyallolella*, and *Pontoporeia*; daphnids such as *Daphnia magna* and *Daphnia pulex*; the mayfly *Hexagenia*; and various oligochaetes. Dr. Chapman said he preferred *Hyallolella* over *Gammarus* because its life cycle is shorter, it is less cannibalistic, and it seems to respond more consistently. *Pontoporeia* appeared to the group to be especially appealing due to its sensitivity to contaminants and its ecological importance in the Great Lakes. As discussed previously, there are amphipod species on both the east and west coasts that are currently being used to assess the potential impact of contaminated salt-water sediments. Dr. Peter Landrum, University of Michigan, was mentioned as a *Pontoporeia* expert and someone who had culturing experience. The culturing of *Daphnia* and its use in toxicity tests are widespread according to the group. It was noted, however, that *Daphnia* is essentially a water-column organism with occasional epibenthic excursions. Dr. Mac reported that he had initially considered using oligochaetes in life cycle tests since a variety of species inhabit Great Lakes sediments and because the oligochaetes are an important benthic ecological component. In addition, the larger oligochaetes are deposit feeders and have enough biomass to conduct bioaccumulation tests. However, they have extremely complex and varied life cycles and reproductive

strategies. As a consequence, the laboratory maintenance requirements have proven to be a substantial obstacle to their widespread culture and use.

43. Dr. Scott explained the term "intrinsic rate of population growth ( $r$ )" and its relationship to life cycle tests. The  $r$  is a numerical value based on a number of observations (e.g., number of progeny produced per female, number of female reproductive days, survival, etc.) made throughout the life cycle. He said  $r$  has been particularly useful in evaluating the effects of contaminated sediments in life cycle studies with saltwater amphipods and mysids. In addition, he indicated that growth, one of the factors contributing to  $r$ , was one of the parameters most often affected. Drs. Adams and Chapman agreed and said they too have often observed growth to be affected during life cycle studies. Other workshop participants agreed that growth was an important and relatively simple measure of biological effect. It was suggested that certain life cycle tests could be shortened considerably by measuring growth and survival during the early life stages of appropriate sensitive species. They cautioned, however, that this shortcut was only appropriate after confirming in replicated full life cycle tests that growth is indeed a good indicator of effects on the entire life cycle. They pointed out that an additional benefit of such an approach is that growth and mortality are relatively straightforward measurements which require minimal training and expertise. This may be an especially attractive feature from a regulatory perspective.

44. Finally, Dr. Scott said that it was very difficult to maintain a quantitative feeding regime during life cycle tests. He said the presence of sediment, suspended and/or bedded, and the fact that some of the species utilized are often sediment ingesters, were both major complicating factors in laboratory tests. All the participants agreed with Dr. Scott's assessment and added that this was particularly crucial since growth, which was just identified as a desirable end point to measure, is directly affected by nutrition. All participants agreed that it would take innovative and imaginative techniques to ensure organisms received adequate quantified rations in tests in which sediments are present.

#### Other bioassessment techniques

45. This session of the workshop was designed to allow participants to discuss techniques that they proposed in the preworkshop inputs but that may



or may not be associated with chronic exposure situations and therefore were not discussed in earlier sessions.

46. Bioenergetics. Dr. Scott briefly explained the bioenergetic endpoints being investigated at the EPA Narragansett Laboratory: scope for growth and net growth efficiency. The former is an instantaneous measure of potential for growth in terms of discretionary or excess energy. The latter is an integrative measure of past physiological conditions, i.e., how efficiently the organism utilized energy available to it during a specific period of time. He has observed significant effects on the bioenergetics of several aquatic organisms exposed to contaminated sediments.

47. Despite these very interesting results, it was pointed out that bioenergetic assessments may not always be appropriate or desirable in a regulatory context. Bioenergetic measurements are generally designed to answer mechanistic questions regarding altered growth or potential for growth. Answering the mechanistic questions may not always be as important to the regulator as simply knowing whether or not growth has been affected. In addition, it takes far less sophisticated equipment and trained personnel to measure a simple change in the organism's mass or dimension over time (i.e., growth) than it does to conduct bioenergetic measurements.

48. Histology/morphology/pathology. Dr. Scott reported that he had seen numerous histopathological effects in a variety of organisms exposed to contaminated sediments. Dr. Dillon noted that it seemed that most published information regarding the histopathological effects of contaminants on aquatic organisms were nonquantitated observations that were highly dependent on who was interpreting the data. Dr. Mac indicated he was aware of research being conducted in which tumors were being induced in a freshwater killifish, the Japanese medaka, within a very short period of time (3 months). The group seemed very interested in the potential for using this fish as a model of tumor formation but noted that further research and refinement prior to its use in a regulatory testing program were required.

49. Sister chromatid exchange. Dr. Scott explained sister chromatid exchange (SCE) to be the unscheduled exchange of genetic material between two sister chromatids of a single chromosome during cell division. This endpoint has been shown to be extremely sensitive and responsive to a number of known mutagens and carcinogens. Small but significantly different SCE rates have been observed in organisms exposed to contaminated sediments in the laboratory

but not in the field. There are two potential drawbacks to using SCE as a bioassessment method. One is the fact that highly trained personnel and sophisticated equipment are required to conduct such an assay. The second has to do with the measure itself. The genetic material that is exchanged between the two sister chromatids is identical. Therefore, it is difficult to interpret the biological importance of altered SCE rates unless supporting corroborative information regarding biological effects is also provided.

50. Ames test. Much of the remaining discussion during this session of the workshop centered on the potential use of the Ames test for determining mutagenic activity and its potential application to sediment testing (Allen, Noll, and Nelson 1983). Dr. O'Connor presented a unique approach he has developed in which differential sediment extracts separated on thin layer chromatography plates are used in conjunction with the Ames test. Observed toxicity and mutagenic activity (or lack thereof) can be associated with very specific sediment fractions. If desired, chemical analysis can be conducted on those portions of the plates in which an effect was observed. The advantage of this approach is that it is rapid, quantitative, and relatively inexpensive. There was a general consensus that it seemed to be a potentially attractive initial screening tool.

51. There was an additional broader general consensus of the group generated during this session. They felt that, at present, there was no single acceptable test that would adequately evaluate the mutagenic/carcinogenic activity of sediments in a regulatory program. However, they felt it was an important area to pursue in light of the public's concern for this category of biological effect.

52. Aryl hydrocarbon hydroxylase induction bioassay. Dr. Mac briefly described the aryl hydrocarbon hydroxylase (AHH) induction bioassay being considered for use by FWS as a screening bioassessment tool. It is designed to signal the presence of potentially hazardous toxic substances that are inducing the hepatic mixed function oxidase enzyme system. Extracts of biological tissue are exposed to rat hepatoma cell lines, and the resultant AHH activity is monitored. In fish at least, it has been shown that AHH activity is positively related to tissue concentrations of polychlorinated organic contaminants (Casterline et al. 1983). The system is calibrated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and results are expressed as TCDD equivalents. There was a consensus that this methodology appeared to be a

promising screening tool but that it should first receive more extensive testing with a variety of aquatic organisms contaminated with a range of environmental contaminants.

53. Adenylate energy charge. The relative concentration of adenylate nucleotides is referred to as the adenylate energy charge (AEC). AEC is a measure of intracellular energy levels, with values greater than 0.80 to 0.85 generally indicating a healthy organism while values less than 0.65 to 0.70 reflecting a deteriorating physiological condition. The numerical quantitation is a very attractive feature from a regulatory perspective. However, the group had various reservations with recommending the use of this particular endpoint since there have been a number of published exceptions to the physiological health status normally associated with specific AEC values. In addition, the methodology requires considerable expertise to prepare tissues for analysis of the adenylate pools and very specialized equipment to detect the nucleotides.

54. Oxygen consumption and osmoregulation. Dr. Alden described his use of oxygen consumption and impaired osmoregulatory ability in aquatic organisms as a screening tool. He said he does not attempt to interpret differences among treatments but rather just identifies that there are differences. He has had substantial success in this approach in the evaluation of contaminated sediments from the Elizabeth River, Va. Sediments taken from a specific portion of this river are very highly contaminated and are acutely toxic. Dr. Alden indicated that he screened sediment samples collected throughout the river using oxygen consumption and osmoregulation responses and was able to pinpoint the most heavily contaminated and toxic portion of the river using these end points.

55. Miscellaneous. A number of bioassessment methods were identified by the workshop participants, e.g., microtox, sea urchin sperm cell motility, phototaxis, burrowing activity, etc. However, there did not appear to be substantial individual or group enthusiasm concerning any one particular test.

### Bioaccumulation

#### Predictive calculations

56. Mr. Rubinstein described research he and others have conducted in which the bioavailability of neutral organic contaminants, such as PCB, is

inversely related to the organic carbon content of the sediment. In addition, ultimate tissue residues are positively related to the lipid content of the organism. He explained that there is a strong theoretical basis for these relationships that is related to molecular thermodynamics. These relationships have been quantitated so that one can calculate the thermodynamically defined maximum bioaccumulation potential (TBP) of neutral organic chemicals, i.e., the maximum possible tissue concentration attainable in an organism of given lipidicity when the only source of contamination is a sediment with a specified organic carbon content and contaminant concentration. In addition, these relationships allow one to examine the kinetics of uptake and, given a minimum of empirical data, calculate the ultimate steady-state concentrations.

57. Mr. Rubinstein suggested a straightforward, three-tiered approach for evaluating the bioaccumulation potential of sediments. Tier I would be a back-of-the-envelope calculation of TBP. This would require data on the concentration of the neutral organic contaminant, the organic carbon content of the sediment, and the percent lipid of the organisms of concern. If the calculated TBP was less than that concentration that would cause concern, then one could omit further testing. If the calculated TBP was of concern, then a tier II laboratory test would be instituted. Mr. Rubinstein suggested a 10-day exposure of animals to sediment. Tissue residue data collected during the test would allow one to examine the kinetics of uptake and to predict final steady-state tissue levels. If these predictive results were acceptable, one could stop at tier II. If not, then a tier III test would be conducted in which final steady-state tissue concentrations were empirically derived.

58. Dr. Adams noted that a very quick nonquantitative way to estimate steady-state tissue concentrations was to look at the concentration of contaminant in the sediment. He has observed that the ratio of tissue concentration to sediment concentration varies from about 0.2 to 2.0 for freshwater organisms. A ratio of 1 would mean that sediment and tissue concentrations were equal. Mr. Rubinstein showed data that indicated a similar range of ratios (from about 0.5 to 1.5) exists for saltwater organisms.

59. Dr. Sullivan noted that an important kinetic consideration is the food conversion factor. He described research at the University of Wisconsin in which fish with different food conversion efficiencies were fed PCB-contaminated food. The results demonstrated that the conversion efficiency, not

percent lipid, was the major factor influencing tissue residues. Fish with lower efficiencies had to process more food and were therefore exposed to more contaminant and had higher residue concentrations.

60. The group noted that the vast majority of research being conducted on predicting bioaccumulation has centered on neutral, hydrophobic, organic chlorinated hydrocarbons such as PCB. Predicting the uptake of petroleum hydrocarbons, which vary greatly in hydrophobicity, is just now receiving attention by the scientific community. It is expected that the predictive tools developed for chlorinated hydrocarbons should generally be applicable to petroleum compounds.

61. Predicting the bioaccumulation of contaminants that are more water soluble (e.g., heavy metals) is much less developed. Unlike neutral organics, which are forced to the sediments due to their hydrophobicity, the nature of metal-sediment interactions is quite different and not well understood. Electrostatic interactions among metal species and sediment molecules are probably the major factors influencing bioavailability. Despite these different factors, the theoretical principles used to develop models for predicting the uptake of neutral organics are also applicable to more water-soluble contaminants. The difficulty lies in identifying the appropriate normalizing factors in sediments and organisms. The group felt that this was an achievable goal for heavy metals but that most resources had, thus far, been devoted to the neutral organics that in general are more toxic and have received greater public attention recently.

#### Organisms

62. The group addressed the question of appropriate organisms to use in assessing bioaccumulation potential. It was noted that bivalves are often used due to their relatively larger size. The major drawback in the use lies in the fact that they are generally insensitive and therefore not good indicators of toxicity during short-term tests.

63. Dr. Adams suggested that, for freshwater evaluations, the laboratory test system he described earlier with the chironomid life cycle may be appropriate. Bioaccumulation could be determined in fourth instar larvae since they are relatively sensitive to acute toxicity and the life cycle can be completed in 30 to 40 days for chronic sublethal assessments. The group felt Adams' approach was a desirable one since it also evaluated acute toxicity and life cycle effects in another organism, *Daphnia magna*. Moreover,

contaminant partitioning among biological and physicochemical components could be determined.

64. Dr. Mac indicated that he had success in using the earthworm *Lumbricus terrestris* in aquatic sediment tests. The group was skeptical of using a terrestrial animal in a submerged soils test. However, Dr. Mac indicated that in his exposure systems the worms actively burrow, exhibit no abnormal behavior, and survive very well. In addition, they provide adequate amounts of tissue for chemical analysis.

#### Gut purging

65. There was considerable discussion on whether animals should be allowed to purge the contents of their digestive tract prior to removal for chemical analysis. Mr. Krauser said the New York District required gut purging in bioaccumulation studies since often the sediments they test are highly contaminated and would bias, in an upward direction, tissue analysis results. However, some participants noted that in many published reports the depuration curves indicated a very rapid loss of contaminants in the early stages of depuration. They noted that gut-purging times often ranged from 1 to 2 days, which was sufficient time to potentially affect resultant tissue concentrations. It was also pointed out that the mass of sediment in the digestive tract may substantially bias, in a downward direction, weight-specific tissue concentrations, especially in smaller animals.

66. There was a consensus by the group that the decisions to utilize gut purging and for how long should be handled on a case-by-case basis. It was pointed out that if the tissue residues are to be interpreted in terms of trophic transfer, then gut purging may not be appropriate since prey organisms are not gut purged prior to ingestion by predators.

#### Trophic transfer

67. Mr. Rubinstein initiated this discussion by reviewing a simple food chain study that clearly documented dietary accumulation in a fish feeding on a worm which had accumulated PCBs from contaminated sediments (Rubinstein, Gilliam, and Gregory 1984). He was careful to note the difference between trophic transfer and biomagnification, the latter term referring to an increase in tissue residues through successive trophic levels. He also explained the difference between bioconcentration and bioaccumulation, the former referring to water-mediated uptake and the latter to uptake from a combination of water and food and/or sediment.

68. Mr. Rubinstein felt that trophic transfer studies could be useful to regulatory personnel if there was a biological resource in or adjacent to the disposal area that could be affected if trophic transfer was a major route of contamination. Dr. O'Connor said that trophic transfer was an extremely complex subject and laboratory designs should be carefully conceived. His preference was for simple sediment-invertebrate-fish designs. Mr. Rubinstein noted that a subsample of invertebrate tissue taken at the end of a sediment bioaccumulation test could be saved for later trophic transfer studies, if warranted.

#### Interpretation of tissue residues

69. Everyone in the group said they had experienced various degrees of frustration in interpreting the biological importance of tissue contaminant levels. Mr. Krauser said New York District regulates to prevent further degradation by comparing residue concentrations obtained in bioaccumulation tests with average concentrations existing in animals in the New York Bight Apex. Unfortunately, there is little generally accepted guidance regarding the ecological importance of specific tissue residues.

70. Dr. Dillon indicated WES had recently published a review of the literature that examined the relationship between sublethal biological effect and tissue residue (Dillon 1984). He indicated that although there were a substantial number of papers reporting biological effects due to contaminant exposure, a very small percentage also contained data on analysis of the tissues. Partly because of this small database, the information was too variable to make specific recommendations.

71. There is some guidance available to interpret contamination levels in aquatic organisms in terms of potential human health hazard. The Food and Drug Administration (FDA) has published tissue concentrations (action levels) for methyl mercury and 10 organochlorine compounds. When concentrations in the edible or whole tissues of fish or shellfish rise above this action level, a potentially hazardous condition is thought to exist for humans.

72. The users at the workshop said that any published numerical value is attractive for their decisionmaking activities. However, they said they realized that FDA limits have been set for an extremely small number of contaminants (11), only one of which is a heavy metal and none of which are petroleum hydrocarbons.

## Consensus Tiered Testing Program

73. In the final session of the workshop, the participants were asked to consider the week's discussions and to arrange the bioassessment methodologies in a tiered testing program they would implement if they were in a position of regulatory authority. Each participant was given written instructions shown in Figure 2. Their individual responses appear in Appendix D. These inputs were rapidly reviewed at the workshop and compiled into a strawman tiered testing program by Dr. Dillon. This strawman (Figure 3) was used to elicit final comments and recommendations from the workshop participants.

74. Tier I activities include those items that one normally initially seeks to discover concerning any dredging and disposal operation. Activities conducted under this tier generally do not involve the generation of new data. Tier II tests consist primarily of screening methodologies designed for rapid assessments. More sophisticated chronic types of bioassessment methodologies are conducted within tier III. These tests are generally more intensive and expensive, but provide more comprehensive, detailed information. If the scope of the dredging project is such that considerable questions remain after tier III testing is completed, or if the project is especially controversial, one may elect to conduct tests such as those listed in tier IV. The group felt these tier IV tests would be most useful when viewed in conjunction with results obtained from tests conducted in earlier tiers.

75. There was a general consensus that, while some of the assessment methodologies in all tiers still required further refinement, there are suitable, technically sound tests currently available for the regulatory evaluation of sediments scheduled for open-water disposal in freshwater environments. There were two items, however, the group felt would be critical in any tiered testing program.

76. The first involves species selection. Since interspecific variation in sensitivity can be considerable, selection of test organisms would greatly affect the results generated. Selection of standard surrogate species as opposed to nonstandard organisms would affect results obtained for individual sediment tests as well as affect the type of database amassed over time for different sediments. Finally, species selection could have a considerable impact on time and cost savings if the species selected could be used in more



18 April 1985

Workshop Participant: \_\_\_\_\_

Below is a list of subjects, including bioassessment techniques, that were discussed during the workshop. Please arrange the items in a tiered testing hierarchy for a regulatory testing program. Items appearing within each tier should be prioritized numerically.

This is your opportunity to provide specific input to the workshop. Comments are strongly encouraged regarding your rationale for the tier, on the individual items, and on any aspect of the workshop.

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Acute Lethality Tests	Histopathology
Adenylate Energy Charge	Life Cycle Tests
Ames Test	Microcosms
Bioaccumulation	Sister Chromatid Exchange
Bioenergetics	Trophic Transfer
Other Bioassessment Tests	

Figure 2. Instructions for preparing suggested tiered testing program

<u>Tier</u>	<u>Activity</u>
I	- Initial assessment: Historical inputs, siting, identification of existing data, etc.
II	- Bulk chemistry - Predictive calculation of bioaccumulation potential (rapid) *- Acute lethality - Ames test (rapid)
III	*- Life cycle test (growth and reproduction) *- Laboratory determination of bioaccumulation potential
IV	- Other bioassessment techniques Bioenergetics, histopathology, AHH, SCE, AEC, microcosms - Trophic transfer potential *- Laboratory determination of steady-state concentrations and important factors affecting bioaccumulation

\*These tests could conceivably be combined into a single test.

Figure 3. Consensus tiered testing program for evaluation of sediments scheduled for open-water disposal in freshwater environments

than one test (e.g., acute toxicity, life cycle studies, and bioaccumulation assessments).

77. The second item the group felt would be critical was the ability to express results from the different tests conducted at one tier in such a manner so as to quantify the decision to go or not to go to another tier. Several participants said that in order to quantitatively express the results of the tests, it would first be necessary to clearly state the objectives of the regulatory program with regards to environmental protection. For example, a policy of no further degradation would dictate a very different set of decisionmaking criteria than a policy designed to eventually return an area to pristine conditions.

#### Workshop Evaluations

78. After the consensus tiered testing program was discussed, the participants were asked to evaluate the workshop by filling out the form shown in Figure 4. As judged by the individual comments (Appendix E) and the numerical summary of the workshop evaluations, the participants considered the workshop a success.

Evaluation Factor*	Numerical Summary of Responses		
	Mean	Range	n
Purpose and objectives of the workshop were clearly stated 5 4 3 2 1	4.5	4-5	14
Objectives could have as easily been met by conducting a thorough literature review 5 4 3 2 1	1.4	1-2	14
Structure of the workshop (i.e., initial short presentation followed by round table discussion) was the best format to achieve the objectives 5 4 3 2 1	4.4	4-5	14
Objectives of the workshop were met 5 4 3 2 1	4.4	3-5	14
No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour) 5 4 3 2 1	4.2	3-5	14
Workshop was worth the trip to Milwaukee (please be honest and disregard the influence of food, drink, and local color) 5 4 3 2 1	4.6	2-5	14
Workshop was worth the trip to Milwaukee (please be honest and include the influence of food, drink, and local color) 5 4 3 2 1	4.8	3-5	11

\* Statements were answered by circling a number from 1 to 5 with 5 equated to a complete "Yes" and 1 to a complete "No"

Figure 4. Workshop evaluation form given to participants. Numerical summary of participants indicated by mean, range, and number of participants responding (n). Individual responses are in Appendix D

### PART III: SUMMARY OF MAJOR AGREEMENTS

79. The following items summarize the major agreements reached by workshop participants:

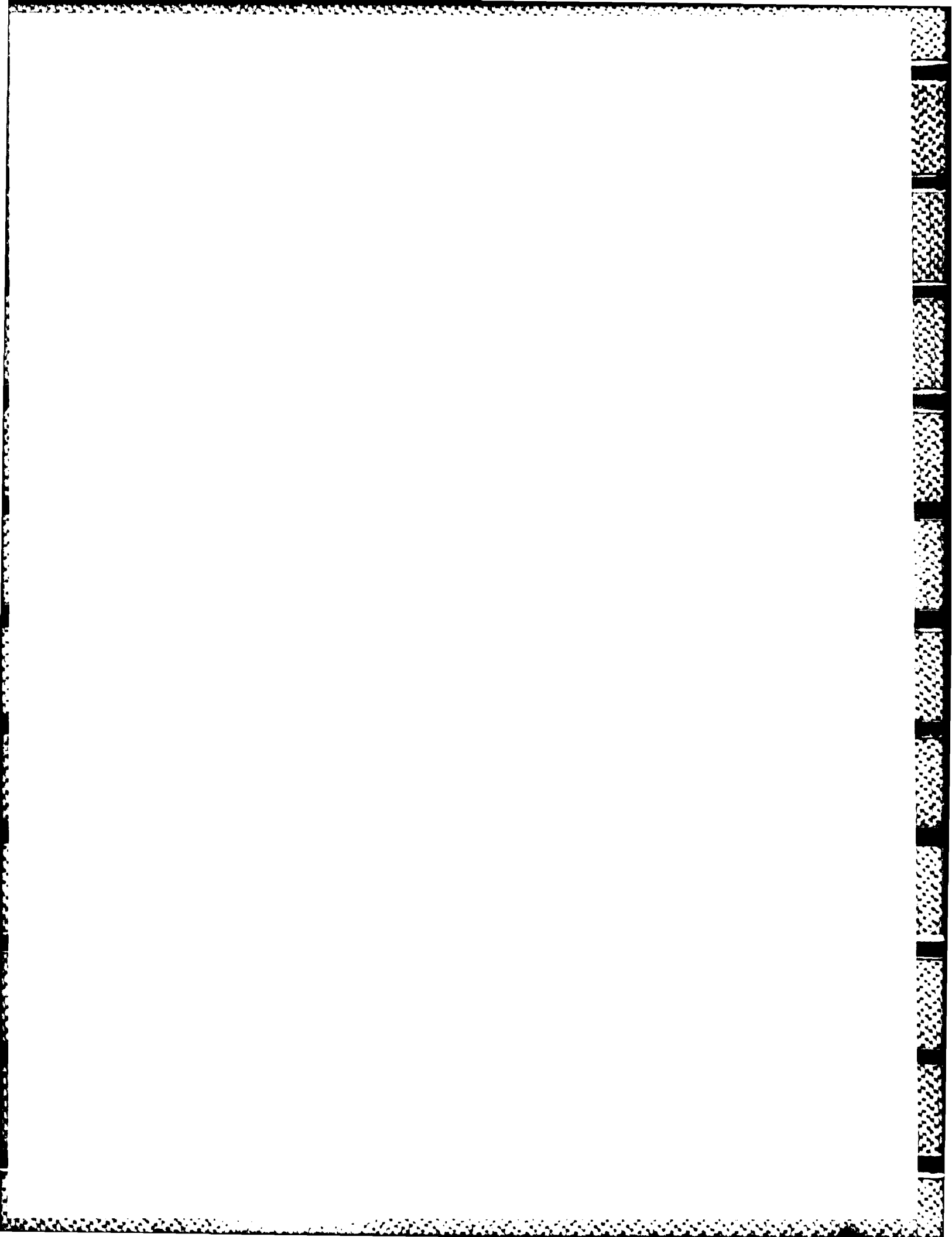
- a. A tiered (hierarchical) testing approach that utilizes tests of increasing complexity and sophistication to reach decisions of greater confidence represents a defensible and technically sound rationale for regulatory decisionmaking.
- b. Bulk sediment chemistry analysis gives no indication of bio-availability and therefore no indication of potential for biological impact. Its primary value lies in providing a qualitative listing of contaminants present in the sediment. One may also use bulk analysis data to calculate the predicted thermodynamically defined maximum bioaccumulation potential for neutral organic chemicals, assuming specific minimal types of data are gathered during the bulk analysis.
- c. Conducting liquid phase acute lethality tests is generally not necessary while solid phase tests should always be carried out. In laboratory tests, the whole sediment (solid and suspended phases) should be considered in experimental design and data interpretation.
- d. A matrix of organisms, as opposed to single species testing, should be used. It should include animals living in sediment (amphipods and larval chironomids and mayflies, etc.) as well as those associated with the benthic substrate (mysids, daphnids, fish, etc.). Ideally, species selected for acute lethality testing should also be able to be used in sublethal and bioaccumulation assessments.
- e. Any regulatory testing program would benefit by the routine use of standard reference toxicant bioassays to assess the sensitivity of test organisms.
- f. The decision to use site water and flowing water exposures in biological tests should be made on a case-by-case basis, but the first consideration must always be meeting the needs of the test organism.
- g. Bioaccumulation of contaminants may be evaluated at three different tiers: (1) mathematical calculation of thermodynamically defined maximum, (2) short-term laboratory tests to indicate the potential for uptake and the prediction of steady-state levels, and (3) long-term laboratory tests to empirically determine steady-state concentrations and the factors affecting bioaccumulation.
- h. The decision to purge the guts of organisms following their removal from sediments must be made on a case-by-case basis. Gut purging should not be used in trophic transfer studies.
- i. Bioaccumulation, bioconcentration, biomagnification, and trophic transfer are complex interactive processes that are

difficult to test in the laboratory and impossible to accurately separate and identify in the field. Laboratory trophic transfer studies, which are simple and therefore easy to decipher, may be desirable if there is an important predator-prey relationship existing in or near a disposal area.

- j. Life cycle tests in which growth and reproduction are determined should receive the highest priority of all nonlethal bioassessment methodologies.
- k. Some sort of oncological assessment is highly desirable in light of the public's concern for this issue. Although there are some potentially useful assessment methods that are in various stages of development and refinement (e.g., modified Ames test, aryl hydrocarbon hydroxylase induction, and tumor induction in medaka killifish), there is no generally accepted test currently available for routine regulatory testing.
- l. There are a number of other bioassessment methods that may be considered on a case-by-case basis. Although they probably would not be used on a routine basis, they could be used when additional biological evaluations are warranted.
- m. A consensus tiered testing program incorporating acute and chronic bioassessment tests as well as methods to determine bioaccumulation potential was developed by the workshop participants. Lacking in this hypothetical testing program are the quantitative keys that dictate at what point one moves from one tier to the next. The formulation of these decision criteria would be driven by the more general decision made by local authorities of what level of environmental protection is desired (e.g., some degradation is acceptable, no further degradation, or a return to pristine conditions).

## REFERENCES

- Allen, H. E., Noll, K. E., and Nelson, R. E. 1983. "Methodology for Assessment of Potential Mutagenicity of Dredged Materials," Environmental Technology Letters, Vol 4, pp 101-106.
- Batac-Catalon, Z., and White, D. S. 1982. "Creating and Maintaining Cultures of *Chironomus tentans* (Diptera:Chironomidae)," Entomological News, Vol 93, pp 54-58.
- Cairns, J., Jr., Dickson, K. L., Maki, A. W. 1978. "Estimating the Hazard of Chemical Substances to Aquatic Life," ASTM STP 657, American Society for Testing and Materials, Philadelphia, Pa.
- Casterline, J. L., Jr., Bradlaw, J. A., Puma, B. J., and Ku, Y. 1983. "Screening of Freshwater Fish Extracts for Enzyme-Inducing Substances by an Aryl Hydrocarbon Hydroxylase Induction Bioassay Technique," Journal Association of Official Analytical Chemists, Vol 66, pp 1136-1139.
- Dillon, T. M. 1984. "Biological Consequences of Bioaccumulation in Aquatic Animals: An Assessment of the Current Literature," Technical Report D-84-2, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Lee, D. R. 1980. "Reference Toxicants in Quality Control of Aquatic Bioassays," Aquatic Invertebrate Bioassays, ASTM STP 715, A. L. Buikema, Jr., and J. Cairns, Jr., eds., American Society for Testing and Materials, Philadelphia, Pa., pp 188-199.
- Nebeker, A. V., Cairns, M. A., Gakstatter, J. H., Malueg, K. W., Schuytema, G. S., and Krawczyk, D. F. 1984. "Biological Methods for Determining Toxicity of Contaminated Sediments to Invertebrates," Environmental Toxicology and Chemistry, Vol 3, pp 617-630.
- Rubinstein, N. I., Gilliam, W. T., and Gregory, N. R. 1984. "Dietary Accumulation of PCB's from a Contaminated Sediment Source by a Demersal Fish (*Leiostomus xanthurus*)," Aquatic Toxicology, Vol 5, pp 331-342.



APPENDIX A: PREWORKSHOP CORRESPONDENCE AND DOCUMENTATION





State of Wisconsin

DEPARTMENT OF NATURAL RESOURCES

Carroll D. Besant  
Secretary

BOX 7121

MADISON, WISCONSIN 53707

File Ref 8250

September 26, 1984

Mr. Louis Kowalski  
U.S. Dept. of the Army  
Corps of Engineers  
Planning Division  
1135 U.S. Post Office  
St. Paul, MN 55101

Dear Mr. Kowalski:

Attached is a summary of the Scope of Work for Management Alternatives for Contaminated Sediment. On June 14, 1984 the State of Wisconsin requested this study as a top priority for FY '85 under Section 22 of the Water Resources Development Act of 1974.

A three-phased approach will: (1) evaluate the toxicity of in-place and resuspended sediment via bioassay techniques; (2) evaluate management alternatives, including (a) leave sediments until buried by clean sediments, (b) remove sediments via dredging; and (3) define the movement of sediments.

Mr. Jack Sullivan, Bureau of Water Resources Management, Surface Water Standards and Monitoring Section will work with your staff to refine this scope of work and to develop a time table and the cost estimate associated with the attached tasks. A meeting on Wednesday, October 10 at 1:00 p.m. in Room 217 of GEF II, here in Madison, will provide an opportunity for DNR staff to review this proposal with your representatives. I would appreciate it if you would include Mr. T.M. Dillon or Dick Petticord, Vicksburg; Mr. Frank Snitz, Detroit; and Mr. Dick Beatty, St. Paul Districts in this meeting. They have all been working together with DNR staff on the Sheboygan and Mississippi Rivers as part of the state's effort to deal with this issue.

If you have any questions concerning this study, please contact me, at the above address, or call me at 608/266-2576.

Sincerely,  
Bureau of Water Resources Management

*Rahim OghaYai*  
Rahim OghaYai  
Water Resources Planning and Policy Section

RO:djc

Enc.

cc: *John*  
Jack Sullivan - WRM/2  
M. Llewelyn - WRM/2  
Scott Hausmann - WRZ/5  
Mary Ann Heidemann - SW/3  
Frank Snitz - COE/Detroit  
Dick Beatty - COE/St. Paul

D. Schuettpelz - WRM/2  
Russell Dunst - TS/2  
Paul LaLiberte - WC Dist., Eau Claire  
Joe Wanielista - COE/Detroit  
→ T. Dillon - COE/Vicksburg  
Dick Petticord - COE/Vicksburg

## I. Project Description

In the last few years it has become apparent that existing techniques for evaluating the toxicity of dredge spoils fall woefully short of providing decision makers with adequate data to choose dredging techniques or ecologically sound disposal options. Furthermore, certain data on sediment pollution exists in Corp of Engineers files, but is not easily accessed by various user groups because of its present format.

The Wisconsin Department of Natural Resources feels that these shortcomings can be overcome through application of Section 22 funded research. A dual approach with separate objectives for each approach is proposed. Year one (phase one) would involve two work efforts. First, a literature and current research review of biological screening techniques that can be used to evaluate the toxicity of sediments. This review should emphasize biomonitoring techniques, however, the review should not be limited to this type of testing. At the same time, and in parallel with the desk top biomonitoring review, certain existing bulk sediment analysis data should be published in report format and made more easily available to various user groups. The specific data targeted for publication is the bulk sediment analysis data generated by the Corp of Engineers, St. Paul District, between 1974-82 from Pools (1-10) of the Mississippi River. It is anticipated that this secondary effort could be completed in one year. (See Figure 1).

Year two (phase two) would involve the testing of the best biomonitoring test methods by various laboratories. Following completion of the testing, an evaluation of the methods by the CUE and Wisconsin Department of Natural Resources (WDNR) would be carried out. After the final method is chosen, year three (phase three) can begin. This phase will involve rigorous testing of the chosen method. A wide variety of sediments qualities should be run to access the ability of the test for widespread application.

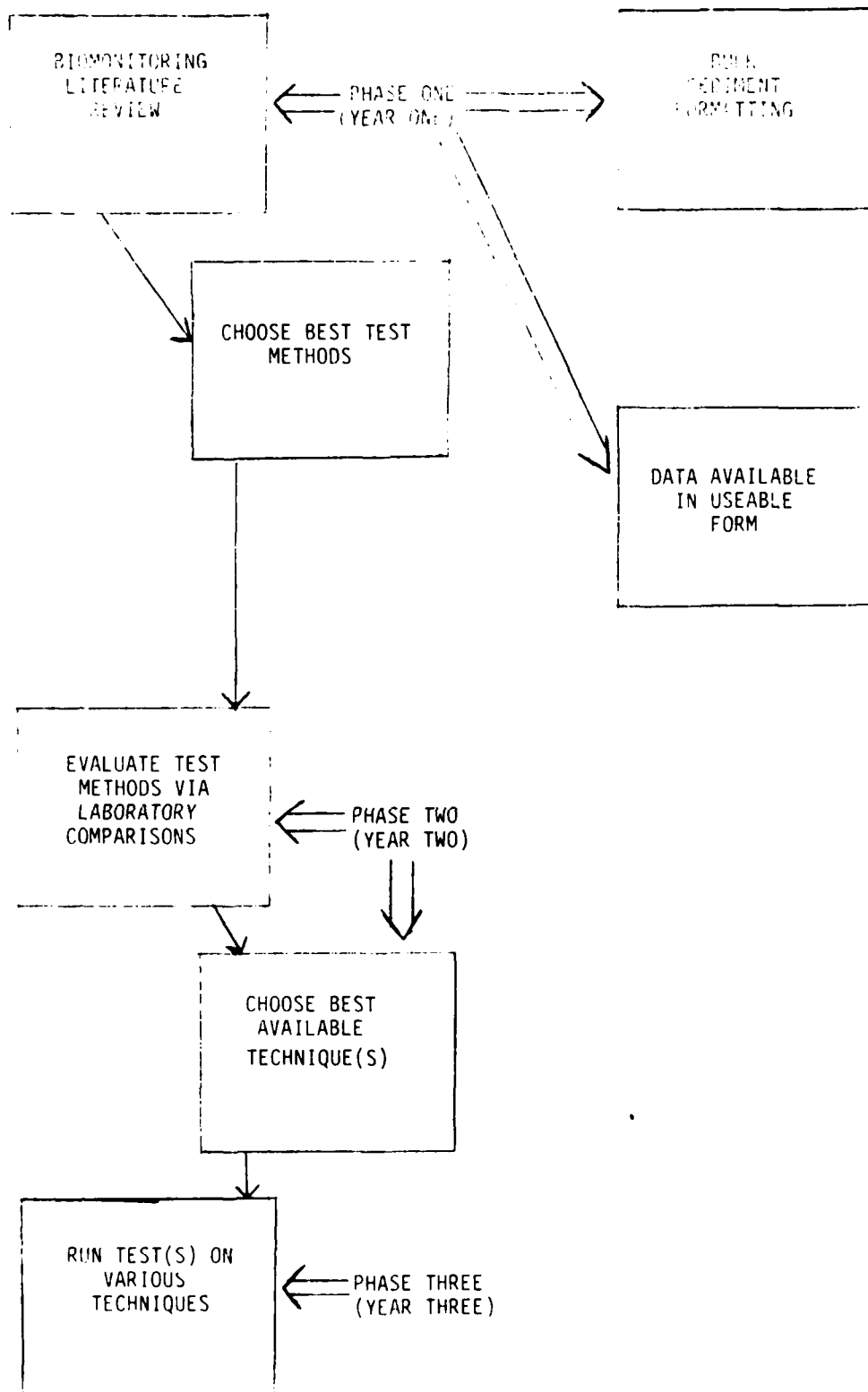


FIGURE 1  
FLOW CHART OF PROPOSED WORK SCHEDULE

## Objectives

### Phase 1 (Year 1) (Task 1)

- To evaluate all available sediment biomonitoring techniques.
- Through joint review select best test methods for laboratory and/or field evaluation.

### Phase 1 (Year 1) (Task 2)

- Make available to all user groups bulk sediment data generated by the COE for pools (1-10) of the Mississippi River.
- Use this data to set monitoring priorities for the Mississippi River.
- Complete this task in year one.

### Phase 2 (Year 2)

- Evaluate tests selected for utility to assess sediment toxicity and ease of use for decision making.
- Select the best biological method available for further testing.

### Phase 3 (Year 3)

- Exhaustively test this procedure on a wide range of sediment types and of varying quality.
- Adopt test procedure as standard protocol to be run for every proposed sediment dredging project.

5140R

## MEMORANDUM FOR RECORD

SUBJECT: Trip Report, Madison, WI

1. At 1:00 p.m. on 10 Oct 84 I met with Rahim Oghalai, John Sullivan, Scott Hausman, Joe Ball, Paul LaLiberte and Ms. Mary Ann Heidmann of the Wisconsin Department of Natural Resources (DNR); Messrs. Stan Kummer and Dennis Anderson of the St. Paul District and Messrs. Frank Snitz and Gary O'Keef of the Detroit District, at DNR headquarters in Madison, WI. Following is a summary of the meeting.
2. Sullivan and Hausman reviewed the DNR Scope of Work recently sent to St. Paul District requesting District cooperation under Section 22 of the Water Resources Development Act of 1974 (PL93-251).
3. Mr. Kummer indicated that Mr. Anderson had recently compiled and programmed all bulk sediment data requested by DNR and this would be made available.
4. Mr. Sullivan indicated that the DNR was hoping WES would be able to assist the State in achieving Task 1 of their Scope of Work, i.e., identification of technically sound bioassessment methodologies. They referred to a recent WES Technical Report (D-84-2, "Biological Consequences of Bioaccumulation in Aquatic Animals: An Assessment of the Current Literature") and hoped we could develop something similar for the DNR but tailored specifically to the situation in Wisconsin. I indicated that we would be willing to assist the DNR in any way possible. However, I made it clear that our primary mission was to support the Corps Districts and that the ultimate decision of tasking out work would fall to Mr. Kummer as the District's Section 22 coordinator. Mr. Kummer indicated he would appreciate the WES input on this matter in whatever manner possible.
5. To achieve Task 1, I suggested that instead of a literature review per se, a workshop be held. I made this suggestion for two reasons. One, much of the information being sought is not published but rather lies in the experience and expertise of individuals and small groups of individuals. Second, a workshop format, with the proper preparation could be accomplished in a much shorter timeframe than a review and interpretation of the published literature.
6. The workshop concept to achieve Task 1 was well received by everyone. I indicated that, pending approval at WES, I would work with Mr. Kummer to initiate the steps necessary to convene the workshop.
7. Other than the initial overview by Messrs. Sullivan and Hausman, there was little time left for discussion of details of the work to be conducted in the

WESES-R

31 Oct 84

SUBJECT: Trip Report, Madison, WI

outyears. The DNR representative did indicate however, that they foresaw the potential for WES involvement throughout the program.

8. On a more general note, Mr. Hausman indicated that he felt within several years, the present Wisconsin statute prohibiting open water disposal of solid material would be repealed or at least significantly altered. This was a significant finding since there are great volumes of dredged material (much of which contains little or no environmental contaminants) removed annually from the upper Mississippi River. While discussing the possibility of disposal alternatives, I explained the Field Verification Program. In addition, I briefly outlined the management strategy which we are developing for Seattle District.

9. The meeting was adjourned at 4:30 p.m.

TOM DILLON  
Research Biologist



DEPARTMENT OF THE ARMY  
WATERWAYS EXPERIMENT STATION, CORPS OF ENGINEERS  
P. O. BOX 631  
VICKSBURG, MISSISSIPPI 39180  
November 19, 1984

REPLY TO  
ATTENTION OF

Environmental Laboratory

Mr. Louis Kowalski  
U. S. Army Engineer District,  
St. Paul  
Planning Division  
1135 U. S. Post Office  
St. Paul, MN 55101

Dear Mr. Kowalski:

Enclosed is a tentative Scope of Work/Discussion Document requested by Mr. Stan Kummer. It describes work to be conducted by the Waterways Experiment Station in response to Wisconsin Department of Natural Resources (DNR) request for assistance under Section 22 of the Water Resources Development Act of 1974.

If you have any questions regarding this matter, please feel free to call me at (FTS) 542-3922. I look forward to assisting the St. Paul District in any way necessary to meet the DNR request.

Sincerely,

Encl

Thomas M. Dillon  
Physical Scientist

HYDRAULICS  
LABORATORY

GEOTECHNICAL  
LABORATORY

STRUCTURES  
LABORATORY

ENVIRONMENTAL  
LABORATORY

COASTAL ENGINEERING  
RESEARCH CENTER

1 March 85

Scope of Work

Submitted to

U.S. Army Engineer District, St. Paul

by

Environmental Laboratory

U.S. Army Engineer Waterway Experiment Station

Vicksburg, Mississippi



## Workshop to Evaluate Sediment Bioassessment Techniques

I. Background: Recently the Wisconsin Department of Natural Resources (DNR) formally requested assistance from the U. S. Army Engineer District, St. Paul, (hereafter referred to as "District") in planning management alternatives for contaminated sediments under Section 22 of the Water Resources Development Act of 1974 (Incl 1). On 10 Oct 84, a meeting was held to discuss how to proceed with the work proposed by the DNR (Incl 2). At that meeting, the Environmental Laboratory (EL) of the U. S. Army Engineer Waterways Experiment Station (WES) suggested that in lieu of a formal literature review to accomplish the objective of evaluating sediment bioassessment techniques (see task 1, year 1, Incl 1) a workshop be convened and attended by technical experts and regulatory personnel with experience in these matters. This preliminary scope of work describes the EL participation in the conduct of such a workshop.

The EL suggested the workshop approach to accomplish the stated objectives for several reasons. Most of the information sought by the DNR does not appear in the open literature per se. Rather, it resides primarily in those persons who have direct experience in conducting regulatory tests with sediments and those who have had to utilize those data in a decision-making environment. In addition, the information on testing methodologies which does appear in the literature rarely discusses how those tests perform in a regulatory context. Another cost-effective advantage of the workshop format is the interaction and immediate feedback among the participants. This important and productive feature is lost in a literature review or even telephone interview approach. The feedback aspect is also especially attractive because it allows the discussions and hence the ultimate output of the workshop to be closely tailored to the specific needs and goals of those convening the workshop. Finally, it has been

the EL experience that any consensus reached by a gathering of highly regarded technical experts generally carries a greater degree of technical credibility than an exhaustive literature review.

This Scope of Work describes a workshop to address the subject of dredged material bioassessment techniques appropriate to meet regulatory needs in Wisconsin. Several other related but distinct efforts for generally similar purposes are ongoing around the country. The workshop participants have been carefully selected to provide relevant information from these efforts, which will be built upon while carefully avoiding duplication. For example, the Dredging Subcommittee of the International Joint Commission on the Great Lakes (IJC) is conducting an evaluation of dredged material assessment techniques. That effort is more research oriented, involves more types of dredging and disposal methods, emphasizes different contaminant and species concerns, and includes a broader scope of evaluative techniques than is appropriate to meet the objectives of Wisconsin as stated in its request for Section 22 assistance. However, to assure optimum coordination of the IJC efforts without duplication, attendees at the workshop have been carefully selected to include IJC participants from the Waterways Experiment Station (WES), U. S. Fish and Wildlife Service and Wisconsin Department of Natural Resources (DNR). Even though IJC goals, objectives, and approach differ, and differ substantially in some respects, from those of the workshop, these participants will assure that IJC efforts contribute optimally to the workshop.

A comprehensive dredged material management strategy and technical decisionmaking framework are being developed for Seattle District and the State of Washington by WES. While these efforts go beyond the objectives of the workshop, they contain much relevant and useful information. The WES participants are well aware of the Seattle work and how appropriate parts of it can

be modified to assist in meeting Wisconsin's objectives. Similarly, WES is assisting Chicago District with site-specific evaluations and demonstration projects involving Indiana Harbor. These also differ in purpose and scope from the workshop, but will provide some useful input through the WES participants. Over the years the New York District has worked extensively with both WES and EPA to develop, refine, and implement a dredged material regulatory program based on bioassessments. Individuals involved in this work from all three agencies will participate in the workshop to ensure that experience gained there is made available to Wisconsin and the District.

All the above efforts are ongoing, none have been fully documented in writing, and many aspects of their practical utility will never be described in reports. Therefore, a workshop forum is the only way to capitalize on what they can offer to Wisconsin and the District. In this way appropriate input from all can be selected and built upon to maximize utility to the workshop sponsors and minimize repetition of both mistakes and learning experiences of others.

II. Approach: The EL will work with the DNR, through the District, to insure the needs of all parties will be addressed. The DNR in conjunction with the District, will provide the EL with the information necessary to clearly define the scope of the workshop and to brief, in writing, potential workshop participants. The EL will develop a list of potential workshop participants agreeable to both the District and DNR. The technical participants will be selected on the basis of scientific credibility and their experience with using bioassessment techniques in the regulatory evaluation of sediments. These participants will be initially contacted by the EL. Participants will be requested to provide to the EL prior to the workshop a selected number of bioassessment

techniques/approaches appropriate for the regulatory evaluation of sediments prior to dredging. They will also be requested to bring with them any and all literature describing advantages and limitations of the techniques they have identified.

The workshop will be conducted in Milwaukee, WI, 16-18 Apr 85. All logistical arrangement will be coordinated through the DNR. In addition to the technical workshop participants, representatives from the DNR, the District, and the EL will be present. The District and DNR participants will be available throughout the workshop to provide input as to the regulatory utility of techniques and approaches under discussion. Since the U. S. Army Engineer District, Detroit, will be involved in the implementation phase (see Phase 3, Year 3, Incl 1), they will be invited to the workshop. Discussion of potentially useful bioassessment techniques will be primarily limited to those suggested by the participants. It is envisioned that discussions may well range into planned outyear activities (see Incl 1). The EL will insure that these discussions will be limited to that which is germane to the objective of the workshop. Near the end of the workshop, the EL will request workshop participants to prioritize the techniques which were discussed and give a rationale for their ranking.

The EL will record minutes of the workshop.

III. Product: Following the workshop, the EL will prepare a proceedings of the workshop. This manuscript will not be a verbatim transcript but rather a detailed summary. It will contain recommendations developed at the workshop which will be useful to the District in fulfilling its Section 22 obligations to DNR. It will also contain a list of references supplied by the workshop participants as well as any other published or unpublished works deemed

appropriate by the EL. The document will be published as an EL Miscellaneous Paper prepared for the District. Unless specifically authorized by the District this report will not contain workshop discussions concerning DNR's three-year plan unless those discussions are directly relevant to the purpose of the workshop. Publication costs to produce this document will be the responsibility of the EL.

IV. Schedule:

<u>Date</u>	<u>Activity</u>
Dec 84	Potential workshop participants identified and contacted
Jan 85	Final selection of participants
Apr 85	Conduct the workshop
May 85	Prepare workshop proceedings
28 Jun 85	Draft report due to the District
31 Jul 85	Comments on draft of proceedings due to the EL
30 Sep 85	Final report due to the District

V. Cost:

Costs associated with all non-Corps personnel will be the responsibility of the DNR.

	<u>WES Budget</u>
One man-month - EL Senior Scientist	6,000
One man-month - EL Technician	4,200
Publication of Workshop Proceedings	4,600

Travel

4 man-trips for WES personnel	4,000
1 man-trip for New England Division personnel	1,000
1 man-trip for New York District personnel	<u>1,000</u>
	20,800



State of Wisconsin

DEPARTMENT OF NATURAL RESOURCES

*Carroll D. Besadny*  
*Secretary*

BOX 7921  
MADISON, WISCONSIN 53707

December 13, 1984

File Ref: 3200

Mr. Tom M. Dillon  
Research Biologist  
U.S. Army Engineer Waterways Exper. Station  
P.O. Box 631  
Vicksburg, MS 39180

Dear Tom:

Enclosed is a brief summary of the recent history of Wisconsin's dredging policies. I think it adequately reflects our current dredging policies and also the difficulties we are encountering in our present decision-making process with respect to chemical contaminants. Also, enclosed is a list of other potential workshop participants that our agency would like invited.

If you have any further questions or comments, feel free to contact me at (608) 267-9753.

Wishing you a Merry Christmas.

Sincerely,  
Bureau of Water Resources Management

A handwritten signature in cursive script that reads 'John R. Sullivan'.

John R. Sullivan  
Surface Water Standards & Monitoring Section

JRS:jm  
Enc.

## Dredging in Wisconsin: A Brief Historical Overview

Concern over open water discharge of polluted dredge material in the Great Lakes began in the late 1960's. In 1968, the EPA Region V Office developed interim guidelines for defining "polluted" dredge material. Upon initiation of the diked disposal program in 1970, the Corps of Engineers (COE) asked the Governors of the Great Lakes states their views on continuing dredging with open water discharge, pending availability of containment facilities. Wisconsin's Governor at that time, Warren Knowles, opposed dumping dredgings in open water under any circumstances. Therefore the COE discontinued maintenance dredging of polluted material pending availability of disposal sites. By chance, during this period there were high lake levels, reducing the impact on navigation of discontinued maintenance.

During the early 1970's, concern mounted over the adverse effects of dredging and disposal operations in the Mississippi River. Legal suits on the dredging issue were filed by the State of Wisconsin against the COE. Eventually, in 1974, a joint organization - the Great River Environmental Action Team (GREAT) - was created to enhance coordinated decision-making regarding dredging practices on the Mississippi. Thus, awareness of the hazards of dredged material disposal had been sparked in the state, so that in 1973, when Wisconsin issued its Wisconsin Pollutant Discharge Elimination System (WPDES), in Chapter 147 of the Statutes, "dredged spoil" was defined as a "pollutant." Because of definition, disposition of all dredged material requires a state permit.

In July 1975, then Governor Patrick Lucey further articulated the state's position regarding open water disposal of dredged material. In a letter to the St. Paul District Office of the Corps of Engineers, the Governor requested that the unannounced disposal of spoils from the Duluth harbor into the open water of Lake Superior be stopped. This letter clarified Wisconsin's prohibition of open water discharge of any dredge material into its adjacent waters. The Governor of Minnesota soon followed suit, and also requested that the Corps cease open water disposal of dredged material in Lake Superior. Based on these requests (and threatened legal action), in-water disposal was ended in Wisconsin Great Lakes waters.

In March 1980, then Governor Dreyfus asked the Wisconsin Coastal Management Council to examine the dredging needs and problems of Wisconsin's Great Lakes Harbors. The Council directed its staff to develop a report on state and federal dredging policies and the status of dredging of Wisconsin Great Lakes Harbors. In October of 1980, the Council established a Dredging Task Force to further examine the issue of harbor maintenance dredging and state and federal regulatory policies. The Task Force was chaired by Wisconsin State Senator Daniel Theno. The Dredging Task Force met several times between January and June, 1981. During these meetings, the current regulatory framework for authorizing dredged material disposal and the economic climate in which Wisconsin's Great Lakes Harbors may find themselves were carefully discussed and evaluated.

At the same time, numerous proposals have been made by the current Federal Administration to charge a substantial portion of the cost of harbor maintenance dredging to state and local governments. Because of this



substantial change in federal policy, there is now a greater concern at the state and local level with the need to hold the cost of harbor maintenance dredging within reasonable bounds without sacrificing environmental quality.

The Task Force concluded that the existing regulatory framework in Wisconsin appears to have sufficient flexibility so that a number of dredged material disposal options could be explored through demonstration projects. The demonstration project approach was desirable because information on the physical and biological impacts and the costs of these disposal options is generally lacking in Wisconsin.

The Council's recommendations were finalized on July 30, 1981, and were subsequently transmitted to Wisconsin Department of Natural Resources Secretary Besadny on August 28, 1981. Deputy Secretary Bruce Braun represented the Department on both the Dredging Task Force and the Coastal Management Council and concurred, on behalf of the Office of the Secretary, in the Council's recommendations. This concurrence means that the department recognizes the value of studying certain disposal options so that more information is available on which to base regulatory decisions. It does not necessarily mean endorsing any in-water disposal option (in fact, on-land disposal is still the preferred approach).

The first round of demonstration projects is intended to deal with the "beach nourishment" disposal option. The intent of beach nourishment is to make use of clean dredged material to replenish material lost from the beach to erosion and to help minimize future erosion. Two projects have been undertaken. The first of these is just east of the Duluth/Superior Harbor. The project placed "unpolluted" (by EPA definition) dredged material near shore in an area just up-drift from a groin field. The shoreline adjacent to an abandoned landfill in this area was experiencing significant erosion and placement of material is expected to help compensate for this loss. The second demonstration project was at Kewaunee, Wisconsin. Clean material dredged from outside the breakwater was deposited along the shoreline south of the harbor to help build up the beach and compensate for past erosion losses which appear to have resulted from the interruption of littoral drift by the harbor breakwaters.

Monitoring studies were done under contract to the Wisconsin Coastal Management Program. Final reports are due early in 1985. However preliminary reports raise major concerns with the predredging sampling and testing techniques.

At present, regulatory decisions must be made on the basis of contaminant specific tests. However, these tests are expensive and may not be conclusive. Regulators still cannot be sure that all significant toxics have been identified, that all significant "hot spots" have been found, or that the presence of a particular toxic poses a significant risk to health or environment which can be avoided or ameliorated by regulation of dredging projects.

The problem of polluted sediments has complicated regulatory decisions on dredging projects, both in Wisconsin and nationwide. More sensitive chemical testing techniques coupled with increased public concern about toxic

materials, have raised tough questions about environmental risks associated with dredging, appropriate dredging methods and the ultimate disposal of polluted dredge spoils.

The Department needs to examine alternative sediment sampling and testing schemes. In contrast to toxic - specific approaches, bioassay and bioaccumulation tests may provide a more effective risk-screening technique, at a lower cost.

2815M

Invitees:

Michael Mac ✓  
USFWS - Great Lakes Fishery Lab.  
Ann Arbor, Michigan

Gary Chapman  
EPA - Sediment Assessment Team  
Corvallis, Oregon

Others:

M. Munawar                      Richard Thomas  
CCIW                              or      IJC  
Burlington, Ontario              Windsor, Ontario

Don Mackay  
University of Toronto  
(416-978-4019)

Gordon Craig  
IEC Beak, Inc.  
Mississauga, Ontario  
416-671-2600

APPENDIX B: PREWORKSHOP INPUTS FROM TECHNICAL PARTICIPANTS

Dr. Bill Adams  
Monsanto Chemical Company  
St. Louis, MO

# Monsanto

ESC-EAG

(CO./DIV./DEPT./LOCATION)

METHOD

(TYPE OF REPORT)

REPORT

REPORT NO.: MSL-4549  
ESC-EAG-M-85-01

JOB/PROJECT NO.: 07-000-760.16

DATE: January 8, 1985

TITLE: A METHOD FOR ASSESSING THE ACUTE TOXICITY OF  
CONTAMINATED SEDIMENTS AND SOILS WITH DAPHNIA  
MAGNA AND CHIRONOMUS TENTANS

AUTHORS: P. S. Ziegenfuss and W. J. Adams

ABSTRACT: A sediment bioassay test has been developed which can be used to assess the aquatic safety of existing or new chemicals that are sorbed to aquatic sediments. This test is applicable to laboratory spiked sediments or contaminated sediments from natural environments. Daphnia magna (<24hrs. old) and Chironomus tentans (10-14 days old) are exposed for 48 hours to six concentrations of test chemical previously sorbed to sediments. Each test includes a control and a solvent control (if applicable). Water quality characteristics are measured at the beginning and termination of each test. Test solutions and test sediments are analyzed for toxicant concentrations at the start and termination of each test if desired. Twenty organisms of each species are exposed to each test concentration for 48 hours. Mortality counts are taken at the end of the test. Data obtained on mortality and toxicant exposure levels are used to calculate the median effect level (LC50 or EC50) for each species for spiked sediments. For naturally contaminated sediments, a comparison of the mortality is made between a control sediment and the sediment of interest. Soil/water partition coefficients are determined from chemical analysis of the test solutions and sediments can be used to predict the toxicity of a neutral organic chemical on a different sediment type.

REPT. NO.: 4549  
AUTHORS: P. S. Ziegenfuss and W. J. Adams  
COPY NO.: 12  
TITLE: A METHOD FOR ASSESSING THE ACUTE TOXICITY OF CONTAMINATED SEDIMENTS  
AND SOILS WITH DAPHNIA MAGNA AND CHIRONOMUS TENTANS

A METHOD FOR ASSESSING THE ACUTE TOXICITY OF  
CONTAMINATED SEDIMENTS AND SOILS WITH DAPHNIA  
MAGNA AND CHIRONOMUS TENTANS

1. SCOPE

- 1.1 This method describes a procedure to determine the acute toxic effects of chemicals sorbed to aquatic sediments to the midge, Chironomus tentans and the daphnid, Daphnia magna, in a single test system over a 48 hour period.
- 1.2 This procedure is applicable to most toxicants which sorb to hydrosoils. However, considerations must be made for compounds which might biodegrade, volatilize, oxidize or photolyze during the test period.
- 1.3 This method may be used to conduct tests with other species of midges and daphnids. However, some modifications may be necessary.

2. APPLICABLE DOCUMENTS

2.1 ASTM Standards

D511	Calcium and Magnesium <sup>a</sup>
D512	Chloride <sup>a</sup>
D516	Sulfate <sup>a</sup>
D857	Aluminum <sup>a</sup>
D888	Oxygen, Dissolved in Water <sup>a</sup>
D1067	Acidity and Alkalinity <sup>a</sup>
D1125	Conductivity <sup>a</sup>
D1126	Hardness <sup>a</sup>
D1129	Definitions <sup>a</sup>
D1179	Flouride <sup>a</sup>
D1193	Reagent Water <sup>a</sup>
D1252	Oxygen Demand, Chemical <sup>a</sup>
D1253	Residual Chlorine in Water <sup>a</sup>
D1293	pH <sup>a</sup>
D1426	Ammonia <sup>a</sup>
D1428	Potassium and Sodium <sup>a</sup>
D1888	Solids, Particulate and Dissolved in Water <sup>a</sup>
D2576	Metals by Atomic Absorption <sup>a</sup>
D2579	Total Organic Carbon <sup>a</sup>
D2872	Arsenic <sup>a</sup>
D3082	Boron <sup>a</sup>
D3086	Pesticides <sup>a</sup>
D3223	Mercury <sup>a</sup>

### 3. SUMMARY

3.1 Chironomus tentans larvae (10-14 days old) and Daphnia magna (<24 hours old) are exposed to uncontaminated control sediments and six concentrations of a test chemical previously sorbed to or spiked on soil or sediment. Fifty grams of sediment and 200 ml of water are placed in a clear glass jar, shaken for 24 hours, and centrifuged at 2000 rpms for 15 minutes. Each test concentration is replicated four times using five daphnids and five midges per replicate yielding a total of 20 organisms of each species per test concentration. The organisms are not fed during the test period. Mortality checks are made at 24 and 48 hours for Daphnia magna, and at 48 hours only for Chironomus tentans. From this data, EC50 or LC50 values and 95% confidence limits are calculated. Determinations of the soil/water partitioning coefficients (Kp) may also be obtained through chemical analysis of the soil and water.

### 4. SIGNIFICANCE

- 4.1 Chironomids and daphnids make up a significant portion of the macroinvertebrate population in many freshwater ecosystems and are an important food source for many aquatic animals. A major change in the population densities of these organisms may have serious adverse effects on ecosystem community structure.
- 4.2 This procedure is designed to assess the acute toxicity of contaminated soils and sediments to D. magna and C. tentans. The burrowing habits of C. tentans insures exposure to sediment and sediment interstitial water, while D. magna are epibenthic grazers and are exposed to the sediment nephroid layer as well as to column water in the test system. The results of a study of this type may be used as part of a hazard assessment program to determine the potential hazard associated with the presence of a chemical bound to soils or sediments.

### 5. DEFINITIONS OF TERMS

- 5.1 Toxicity - Quality, state or degree of harmful effect resulting from an exposure to a toxicant [1].
- 5.2 Toxicity Test - An experimental study designed to measure the degree of harmful effects resulting from an exposure to a toxicant.
- 5.3 Toxic Agent - A substance which kills or impairs health through its chemical or physical action.
- 5.4 Toxicant - A substance (a poison) which, when taken into or formed in the body, kills or impairs health [2].
- 5.5 Static Toxicity Test - A toxicity test in which the dilution water and chemical are mixed together at the beginning of the test and are not renewed during the study.



- 5.6 Stock Solution - A concentrated solution of the toxicant in dilution water or solvent which is used to spike the test soil or sediment.
- 5.7 Test Concentration - The dose or quantity of toxicant to which the daphnids and midges are exposed for 48 hours.
- 5.8 Treatment(s) - Refers to the groups of organisms which are exposed to the toxicant as opposed to the controls.
- 5.9 Test Solution - A mixture of the toxicant and the dilution water resulting from the desorption of the chemical from the sediment into the overlying water.
- 5.10 Sediment Interstitial Water - The water occupying the spaces between sediment particles. Often referred to as sediment pore water.
- 5.11 Sediment Exposure Acute Toxicity Test - A short term (usually 48 hours) exposure of Daphnia magna and Chironomus tentans to a toxicant previously sorbed to soil or sediment to determine the EC 50 or LC50 of the toxicant.
- 5.12 For definitions of other terms in this practice, refer to ASTM Definitions D1129, Terms Relating to Water (1). For an explanation of units and symbols, refer to ASTM Standard E380, Metric Practice Guide (4).

## 6. SAFETY PRECAUTIONS

- 6.1 Many substances may adversely affect human beings if adequate precautions are not taken. Therefore, contact with all toxicants and test solutions should be minimized, and special precautions, such as covering test chambers and increasing ventilation, should be taken with volatile toxicants. Information on toxicity to humans and recommended handling procedures (5) should be studied before tests are begun with any toxicant.
- 6.2 Although disposal of stock solutions, test solutions, and test organisms poses no special problems in most cases, health and safety precautions should be considered before beginning a test. Removal or degradation of a toxicant before disposal of stock and test solutions is sometimes desirable.
- 6.3 Rinsing with acetone and other volatile solvents should be performed only in well-ventilated areas.
- 6.4 Acid and hypochlorite solutions should not be mixed because hazardous fumes may be produced.

## 7. APPARATUS

- 7.1 Facilities. During culturing and testing, test organisms are shielded from disturbances. The test facility must be well ventilated. A 16 hour light and 8 hour dark photoperiod should be provided. Light intensity must be low 200-400 lux (18-36-74 foot candles) at the surface of test solutions and provided by wide spectrum (Color Rendering Index 90) fluorescent lamps.
- 7.2 Test Chambers. Sediment exposure toxicity tests are conducted in clear 250 mL polycarbonate or glass wide mouth jars which contain 200 mL of dilution water.
- 7.3 Cleaning. Test chambers and equipment used to prepare and store dilution water, stock solutions and test solutions must be cleaned each time before use, i.e., see SOP #EAS-80-SOP-003 [3].

## 8. REAGENTS AND MATERIALS

- 8.1.1 A minimal criterion for an acceptable dilution water is that healthy, unfed Daphnia (<24 hours old) will survive in it for 48 hours without signs of stress [6].
- 8.1.2 Commonly used dilution waters are from wells or surface waters and should be uncontaminated and of constant quality and meet the following specifications:
- |   |           |               |
|---|-----------|---------------|
| Particulate matter  | 20        | mg/liter      |
| TOC or COD  | 5         | mg/liter      |
| Un-ionized ammonia  | 20        | ug/liter      |
| Residual chlorine   | 3         | ug/liter      |
| Total organophosphorus pesticides                                     | 50        | ng/liter      |
| Total organochlorine pesticides plus PCBs or organic choorine         | 50        | ng/liter      |
| Hardness (mg/l CaCO <sub>3</sub> )                                    | 25        | ng/liter      |
| pH  | 100       | mg/liter      |
| Boron, fluoride   | 7.0 - 8.2 |               |
| Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc | 100       | ug/liter each |
| Cadmium, mercury, silver  | 1         | ug/liter each |
|   | 100       | ng/liter each |
- 8.1.3 A natural surface water is acceptable and is in fact preferred if the test is to be performed with naturally contaminated sediment. Water from the site of interest would be best.
- 8.1.4 A natural dilution water is considered to be of constant quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than 10 percent of their respective averages and if the monthly range of pH is less than 0.4 units. Only as a last resort will dechlorinated

city water be used. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chloride or chloramines.

8.1.5 Our recommended dilution water is St. Peter's well water. This water has been used for the past several years in the Environmental Sciences Aquatic Laboratory. The history of the water is well documented and it has been shown to be of high quality. Well water is the water of choice because it so closely approximates natural surface waters, is free of chemical contamination and is used in time independent and chronic toxicity tests with acceptable survival, growth and reproduction [7].

8.1.6 Reconstituted Dilution Water. Reconstituted water is prepared by adding specified amounts of chemicals to high quality distilled or ionized water. Reconstituted water is recommended for use when measuring the effects of chemical parameters (pH, hardness, etc.) on the toxicity of a toxicant or for interlaboratory comparative toxicity tests. Reconstituted water can be prepared as described in the reference [7].

## 8.2 TOXICANT

8.2.1. The toxicant should be reagent grade or better, except for tests formulations or commercial products. If the identity and concentration of major ingredients and major impurities are not known, they should be determined. The toxicant should be added to the sediments without the use of solvents if possible. If a solvent is necessary, it must be one which can be driven off (i.e., evaporated) leaving only the test chemical on the sediments.

8.2.2 The stability of the toxicant in the stock solution should be verified by chemical analyses. Stock solution sub samples should be analyzed immediately after the stock has aged the maximum length of time it would be in use in the tests.

## 9. SEDIMENTS

9.1 The sediments used for control organisms or for spiking (dosing) in the Environmental Sciences Laboratories are obtained from local undisturbed agricultural soils. These soils can be used as found or mixed with a high organic carbon (OC) soil such as a composted manure. The sediment chosen for use should be characterized and at least the following should be known: pH, % organic carbon, % sand, % silt, % clay, % water holding capacity, and ionic exchange capacity.

9.2 Sediments from lakes or rivers known to be relatively free of contaminants are acceptable and should be characterized as described in 9.1.

- 9.3 Sediments are prepared for use by heating at 100°C for 12 hours and sieving through a No. 25 U.S. Standard Sieve (710 micron opening). All characterizations should be made after the soil has been heated and sieved.
- 9.4 Sediments which are prone to leach acidic substances into the dilution water should not be used for this testing procedure as the dilution water pH may become too low for the test organisms to survive.

## 10. TEST ORGANISMS

- 10.1 Daphnia magna is the recommended daphnid species because of its large size, ease of identification, availability from laboratories and commercial sources, ease of handling, and past use. Successful tests have also been conducted using both Ceriodaphnia sp. and Daphnia pulex.
- 10.2 Chironomus tentans is the recommended chironomid test species because of its relatively large size, ease of culture and handling and habit of burrowing into sediments to build a case retreat. This method could easily be adapted to other species of midges with similar habits.
- 10.3 The identity of organisms obtained from laboratories and commercial sources should be verified regardless of any information that comes with the organisms, since such information is not always accurate.
- 10.4 Handling. Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible, so that the organisms are not unnecessarily stressed. Organisms that touch dry surfaces or are dropped or injured during handling should be discarded.

## 11. PROCEDURE

- 11.1 Soil Preparation. Soil for this procedure may be spiked with the test toxicant by one of two methods. In the first method, the amount of soil to be spiked is placed in a stainless steel evaporating pan and saturated with excess volatile solvent. The solvent used must readily dissolve the toxicant but not alter the toxicant's chemical structure. Acetone and methylene chloride are commonly used. An appropriate amount of solvent containing the toxicant is used to saturate the soil (1:1, solvent: soil) and form a slurry. The mixture is stirred repeatedly while the solvent evaporates. When all the solvent has evaporated from the soil it is ready for testing. This method, while quick and easy, requires that the soil be saturated with solvent, which may affect the desorption of the toxicant into the dilution water. If this method is used, it is necessary to create a solvent control soil in addition to the regular control by saturating the soil with solvent and evaporating it off without adding toxicant.

- 11.2 A second and preferred soil spiking procedure consists of placing approximately 50 mLs of solvent (with toxicant) in a clean, wide mouth 1-2 L glass jar. Evaporate the solvent by blowing air into the jar with an air hose while slowly spinning the jar on its side. When the solvent is evaporated, the toxicant should be fairly well distributed on the jar walls. The amount of sediment to be spiked (300-400g) is then placed in the jar along with enough dilution water to produce a pourable slurry. The jar is capped and placed on a mechanical shaker for 24 to 120 hours, depending on the physical properties of the toxicant i.e. chemicals with lower water solubilities require longer shaking periods. At the end of this period allow the sediment to settle, pour off the water, and the soil is ready for testing.
- 11.3 Chemicals analysis of the spiked soil should be performed prior to the start of a test. The use of radiolabeled toxicants can facilitate this. Toxicants which volatilize rapidly may not be suitable for use in this method.
- 11.4 After a minimum of five and preferably six concentrations of spiked soils have been prepared, the test may be started. The test is performed in 250 mL clear polycarbonate (or glass) centrifuge bottles (available from fisher scientific, catalogue #05-430-53). Four replicates are used per concentration. Place 50g of soil (dry weight, or dry weight equivalent) in each bottle and add 200 mL of dilution water. If the sediment is collected from the field or is dosed as described in 11.2 do not dry the soil, but use a dry weight equivalent conversion to calculate the amount needed. The bottles are mechanically shaken for 24 hours to facilitate desorption of the chemical from the soil into the water. The bottles are then centrifuged at 2000 rpm (500 xg) for 15 minutes to clarify the column water.
- 11.5 After all the bottles have been centrifuged, the caps are removed, and water chemistry measurements are made (temperature, dissolved oxygen concentration, and pH) [8,9,10]. Water samples can be taken at this time for chemical analysis if radioisotopic analyses are being used.
- 11.6 Test organisms are placed in the test bottles at this time in random order. Midges are added first [11], one midge at a time should be placed in each bottle and be given time to burrow before the next midge is added. This will prevent the midges from attacking each other. Midges used in this test should be between 10 and 14 days old (2nd instar). At the test start five midges are placed in each bottle (20 per test concentration). Five daphnids (<24 hours old) are then added to each bottle [12] in random order.
- 11.7 The bottles are left uncapped and exposed to a 16 hours on/8 hours off light regime of 200-400 lux (18-36 foot candles).

Test organisms are not fed during the test and should be disturbed as little as possible. Daphnid mortality checks are made at 24 hours by counting the living daphnids in each bottle. An organism capable of any movement is considered alive for both species. Organisms which cannot move are considered dead.

#### 11.8 Test Termination

- 11.8.1. A final (48 hour) mortality check is made by removing the daphnids from the test bottles by pipette.
- 11.8.2. Water quality measurements (temperature, dissolved oxygen concentration, pH, alkalinity and hardness) are taken after the daphnids have been removed and before the midges are removed [13, 14].
- 11.8.3. Water samples for chemical analysis are taken after water quality measurements are made.
- 11.8.4. The remaining column water is carefully decanted from the bottles and the midges are picked from the sediment and counted for survival.
- 11.9. Sediment and Interstitial Water Chemical Analysis - The sediment remaining in each bottle after removing the midges is centrifuged at 9000 x g. The resulting supernatant and sediment pellet are then analyzed for toxicant concentration. The soil/water partitioning coefficients (Kp and Koc) may be determined by dividing the sediment chemical concentrations by the water chemical concentration [15]. A calculation of sediment partition coefficient for the overlying surface water in the test system is also possible. A comparison of the interstitial Kp and surface water Kp gives an indication of the equilibrium of the system.
- 11.10. This test method may be used to assess the acute toxicity of soils or sediments from contaminated field sites. No soil spiking is necessary and no dose response data is obtained from such a test. The sediments or soils are used as collected together with field or laboratory control soil samples.

#### 12. Calculations

- 12.1. Test concentrations and corresponding percent mortality data derived from the definitive test are used to calculate the 48 hour median effective concentration (LC50) and its 95% confidence interval. Calculation of the LC50 is done by hand by means of the Litchfield - Wilcoxon method [16] or by a computer program designed to calculate and LC50 by

means of probit analysis, moving averages, and binomial analysis [17]. The method of analysis which determines the LC50 with smallest confidence interval will be the method of analysis reported.

- 12.2 The soil/water partition coefficient is calculated by the following formula.

$$K_p = \frac{\text{chemical concentration of soil}}{\text{chemical concentration of water}}$$

The soil and water chemical concentration must be in like units (i.e. - mg/Kg and mg/L). The carbon normalized partition coefficient is calculated by the following formula.

$$K_{oc} = \frac{K_p}{\% \text{ oc of the soil expressed as a decimal}}$$

### 13. QUALITY ASSURANCE

#### Quality Assurance

- 13.1 Criteria for rejection of a test.

- 12.1.1 More than 10 percent of the Control or Solvent Control die.
- 12.1.2 Temperature deviation from 22°C exceeds 3°C.
- 12.1.3 Dissolved oxygen drops below 40 percent of saturation.
- 12.1.4 pH deviates by more than one pH unit.
- 12.1.5 Fifty percent mortality is exceeded at all test concentrations.

### 14. REPORT

The results reported should include the following:

- 14.1 Name of test, investigator, laboratory, and date test was conducted.
- 14.2 A brief description of the toxicant including its source and lot number or a description of the field contaminated sediment.
- 14.3 The source of the dilution water, its chemical characteristics, and a brief description of any pretreatment.
- 14.4 A brief description of source of Daphnia, their history, experimental design and summary of methods.

- 14.5 Methods used for, and results of, all analysis of test water.
- 14.6 Methods used for, and results of, statistical analysis data:
- 14.7 Anything unusual about the test, any deviation from the protocol and any other relevant information.
- 14.8 Raw data. (See Appendix I)
- 14.9 Data Retention. All original raw data generated in the study will be provided to the Department of Medicine and Environmental Health staff toxicologist, and the Environmental Sciences Assessment Group GLP file. A copy of the Final Report (without raw data) will be sent to the Product Acceptability Manger, Environmental Sciences Manager, Environmental Assessment group leader and authors. Data will be retained in MIC GLP file for ten years.

15. METHOD CHANGES

In the event that modifications of this method are deemed necessary, a written statement of any changes and reason(s) will be provided by the study director. All agreed changes will be expressed in writing, signed and dated by the study director. The signed changes will be appended to the method and included in the final report.



REFERENCES

1. Annual Book of ASTM Standards, Part 31, Philadelphia, PA American Society for Testing and Materials, 1979.
2. Post, G. 1977. Glossary of Fish Health Terms. American Fisheries Society, Fish Health Section, 48 pp.
3. Standard Operating Procedure. MIC Environmental Sciences. Cleaning Culture Tanks and Test Aquaria. Document number EAS-80-SOP-003.
4. Annual Book of ASTM Standards, Part 41, Philadelphia, PA. American Society for Testing and Materials, 1976.
5. For example see: Manufacturing Chemist Association, 1975. Laboratory Waste and Disposal Manual. Washington, D.C. 176 pp.
6. Stephen, C.E., 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-600/3-75-009.
7. Special Study. MIC Environmental Sciences. Water Quality Characteristics of the Water Samples Used in the N-Building Environmental Laboratory Part II. Report number ES-78-SS-22.
8. Standard Operating Procedure. MIC Environmental Sciences. Temperature Measurement. Document number EAS-80-SOP-010.
9. Standard Operating Procedure MIC Environmental Sciences. Dissolved Oxygen Determination. YSI 54ARC Operation, Maintenance and Calibration. Document number EAS-80-SOP-011.
10. Standard Operating Procedure. MIC Environmental Sciences. pH Determination. Document number EAS-80-SOP-077.
11. Standard Operating Procedure. MIC Environmental Sciences. Procedure for Culturing the Midge, Chironomus tentans. Document number EAS-82-SOP-049.
12. Greuber, D. J. and W. J. Adams, 1980. MIC Environmental Assessment Method for Conducting Acute Toxicity Test with Daphnia magna. Document number ES-80-M-6.
13. Standard Operating Procedure. MIC Environmental Sciences. Alkalinity Measurement. Document number EAS EAS-80-SOP-008.
14. Standard Operating Procedure. MIC Environmental Sciences. Hardness Measurement. Document number EAS-80-SOP-009.
15. Michael, P. R. and R. G. Kaley, 1979. A method for the Determination of Soil/Water Partition Coefficients. Report number ES-79-N-19.
16. Litchfield, J. T. and F. Wilcoxon, 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour Pharm. Exp. Ther. 96-99-113.
17. Stephan, C.E. 1978. Personal Communication. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

**Monsanto**

MIC ENVIRONMENTAL SCIENCES  
(CO./DIV./DEPT./LOCATION)

METHOD REPORT  
(TYPE OF REPORT)

REPORT NO.: ES-82-M-7

JOB/PROJECT NO.: 43-000-760.26-8700044

DATE: June 23, 1982

TITLE: METHOD FOR CONDUCTING ACUTE TOXICITY TESTS WITH  
THE MIDGE CHIRONOMUS TENTANS

AUTHORS: R. G. Mosher and W. J. Adams

ABSTRACT: Chironomus tentans larvae 10-14 (2nd instar) days old are exposed to a toxicant for a period of 48 hours. At least 5 concentrations of test chemicals, a control receiving no chemical, and a solvent control (for those chemicals requiring a solvent) are recommended. Each test concentration is replicated 10 times using 1 larva per replicate, yielding a total of 10 larvae per treatment. Controls are treated the same as the treatments except they are not exposed to the toxicant. The larvae are not fed during the testing period. At 24 and 48 hours, survival measurements are made. From this data, LC50 values and 95% confidence limits are calculated for the 24 and 48 hour intervals.

R. G. Mosher and W. J. Adams

METHOD FOR CONDUCTING ACUTE TOXICITY TESTS WITH THE MIDGE  
CHIRONOMUS TENTANS

METHOD FOR CONDUCTING ACUTE TOXICITY TESTS WITH THE MIDGE  
CHIRONOMUS TENTANS

1. SCOPE

- 1.1 This method describes a procedure for obtaining laboratory information on the acute toxicity of chemicals to the midge, Chironomus tentans. The test is conducted under static conditions during a 48-hour period.
- 1.2 This method describes testing procedures using C. tentans, but with some minor modifications could be used for other species of midges.
- 1.3 This procedure is applicable to most toxicants. However, considerations must be made for materials that might biodegrade, volatilize, oxidize, photolyze or sorb during the 48-hour period.

2. APPLICABLE DOCUMENTS

2.1 ASTM Standards

D511	Calcium and Magnesium <sup>a</sup>
D512	Chloride <sup>a</sup>
D516	Sulfate <sup>a</sup>
D857	Aluminum <sup>a</sup>
D888	Oxygen, Dissolved in Water <sup>a</sup>
D1067	Acidity and Alkalinity <sup>a</sup>
D1125	Conductivity <sup>a</sup>
D1126	Hardness <sup>a</sup>
D1129	Definitions <sup>a</sup>
D1179	Fluoride <sup>a</sup>
D1193	Reagent Water <sup>a</sup>
D1252	Oxygen Demand, Chemical <sup>a</sup>
D1253	Residual Chlorine in Water <sup>a</sup>
D1293	pH <sup>a</sup>
D1426	Ammonia <sup>a</sup>
D1428	Potassium and Sodium <sup>a</sup>
D1888	Solids, Particulate and Dissolved in Water <sup>a</sup>
D2576	Metals by Atomic Absorption <sup>a</sup>
D2579	Total Organic Carbon <sup>a</sup>
D2972	Arsenic <sup>a</sup>
D3082	Boron <sup>a</sup>
D3086	Pesticides <sup>a</sup>
D3223	Mercury <sup>a</sup>

<sup>a</sup>1976 Annual Book of ASTM Standards, Part 31.

### 3. SUMMARY

- 3.1 Chironomus tentans larvae 10-14 (2nd instar) days old are exposed to a toxicant for a period of 48-hours. At least 5 concentrations of test chemical, a control receiving no chemical, and a solvent control (for those chemicals requiring a solvent) are recommended. Each test concentration is replicated 10 times using 1 larva per replicate, yielding a total of 10 larvae per treatment. Controls are treated the same as the treatments except they are not exposed to the toxicant.

The larvae are not fed during the testing period. At 24 and 48 hours, survival measurements are made. From this data, LC50 values and 95% confidence limits are calculated for the 24 and 48 hour intervals.

### 4. SIGNIFICANCE

- 4.1 Chironomus tentans and other midges make up a significant portion of the macroinvertebrate community in many freshwater ecosystems and are an important food source for fish and waterfowl. A major change in the availability of these and other macroinvertebrates in aquatic habitats could have serious effects on the structure of the ecosystem community.
- 4.2 This procedure is designed to assess the effects of toxicants on the survival of second instar larvae of C. tentans, a representative macroinvertebrate. The results of a given study can be used as part of a safety assessment program.

### 5. DEFINITION OF TERMS

- 5.1 Toxicity - Quality, state or degree of harmful effect resulting from an exposure to a toxicant [1].
- 5.2 Toxicity Test - An experimental study designed to measure the degree of harmful effects resulting from an exposure to a toxicant.
- 5.3 Toxic Agent - A substance which kills or impairs health through its chemical or physical action.
- 5.4 Toxicant - A substance (a poison) which, when taken into or formed in the body, kills or impairs health [2].
- 5.5 Static Toxicity Test - A toxicity test in which the dilution water and chemical are mixed together at the beginning of the test and are not renewed during the study.
- 5.6 Midge Acute Toxicity Test - A short term (usually 48 hours) exposure of midge larvae to a toxicant for the purpose of determining the LC50 of the toxicant.
- 5.7 Test Concentration - The dose or quantity of toxicant placed in solution in the dilution water to which the midge larvae are exposed for 48 hours.

- 5.8 Treatment(s) - Refers to the groups of midge larvae which are exposed to the toxicant as opposed to the controls.
- 5.9 Test Solution - A mixture of the toxicant and the dilution water in which the midge larvae reside during the study.
- 5.10 Stock Solution - A concentrated mixture of the toxicant and dilution water or carrier solvent which is mixed with dilution water to prepare a test solution.
- 5.11 EC50 Value - Concentration which effects (immobilizes) 50 percent of the test population.
- 5.12 Midge - Common name of a group of mosquito-like flies belonging to the family Chironomidae.

## 6. APPARATUS

- 6.1 Facilities. During culturing and testing, test organisms are shielded from disturbances. The test facility must be well ventilated. A 16 hour light and 8 hour dark photoperiod should be provided. Light intensity must be 400-800 lux (47-74 foot candles) at the surface of test solutions and provided by wide spectrum (Color Rendering Index 90) fluorescent lamps.
- 6.2 Test Chambers. The static toxicity test is conducted in 50 mL beakers which contain 40 mL of test solution.
- 6.3 Cleaning. Test chambers and equipment used to prepare and store dilution water, stock solutions and test solutions must be cleaned each time before use, i.e., see SOP #EAS-80-SOP-003 [3].

## 7. REAGENTS AND MATERIALS

### 7.1 Dilution Water

- 7.1.1 A minimal criterion for an acceptable dilution water is that healthy, unfed midge larvae will survive in it for 48 hours without signs of stress [4,5].
- 7.1.2 Commonly used dilution waters are from wells or surface waters and should be uncontaminated and of constant quality and meet the following specifications:

Particulate matter	<20 mg/liter
TOC or COD	< 5 mg/liter
Un-ionized ammonia	<20 µg/liter
Residual chlorine	< 3 µg/liter
Total organophosphorus pesticides	<50 ng/liter
Total organochlorine pesticides plus PCB's or organic chlorine	<50 ng/liter
	<25 ng/liter

Hardness (mg/L CaCO <sub>3</sub> )	> 30 mg/liter
pH	7.0-8.2
Boron, fluoride	<100 µg/liter each
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc	< 1 µg/liter each
Cadmium, mercury, silver	<100 ng/liter each

7.1.3 A natural dilution water is considered to be of constant quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than 10 percent of their respective averages and if the monthly range of pH is less than 0.4 units. Only as a last resort will dechlorinated city water be used. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chloride or chloramines.

7.1.4 The recommended dilution water is St. Peter's well water. This water has been used for the past several years in the Environmental Sciences aquatic laboratory. The history of the water is well documented and it has been shown to be of high quality. Well water is the water of choice because it so closely approximates natural surface waters, is free of chemical contamination and is used in time independent and chronic toxicity tests with acceptable survival, growth and reproduction [6].

7.1.5 Reconstituted Dilution Water. Reconstituted water is prepared by adding specified amounts of chemicals to high quality distilled or deionized water. Reconstituted water should only be used when measuring the effects of chemical parameters (pH, hardness, etc.) on the toxicity of a toxicant or for interlaboratory comparative toxicity tests. Reconstituted water can be prepared as described by Stephan [4].

7.2 Toxicant. The major components of the toxicant should be known. The toxicant should be added to the dilution water without the use of solvents or other carriers, if possible. If carriers other than water are necessary, the amount used must be kept to a minimum, preferably less than or equal to .5 mL solvent/L test water. Dimethylformamide and triethylene glycol are the preferred organic solvents. Other suitable solvents include methanol, acetone, and ethanol.

## 8. PRECAUTIONS

8.1 Some substances can adversely affect human beings if adequate precautions are not taken. Therefore, contact with all the toxicants and test solutions should be minimized. Information on toxicity to humans and recommended handling procedures should be studied before tests are begun with any toxicant.

## 9. TEST ORGANISMS

- 9.1 Chironomus tentans is a recommended test species due to its ease of culturing, relatively large size as second instar larvae, short time required to raise larvae to second instar and ease of handling the larvae.
- 9.2 Midge larvae will be cultured according to EAS-82-SOP-44 (7).
- 9.3 Handling. Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully and quickly as possible so that organisms are not unnecessarily stressed. Organisms should be introduced beneath the air/water interface. Smooth glass Pasteur pipettes can be used for collecting the midge larvae.
- 9.4 Quality. The quality of the test organisms will be determined by the criteria of acceptability specified in 12.1. If these criteria are not met, the quality of the organisms will be considered unacceptable and the test will be invalid.

## 10. PROCEDURE

- 10.1 Preliminary Preparations. Twelve to 16 days before an acute test is begun, 3 freshly laid Chironomus tentans egg masses are placed in a clean 20x40 cm glass or enameled rearing pan filled with well water to a depth of 3 cm. No substrate is added to the pan. At 20°C, larvae will begin to appear in 48 hours. Food (Tetra Conditioning Food Vegetable Diet suspension) is then added at the rate of .5 mL per day (7). Fresh water is added as needed to make up for that lost to evaporation. The larvae in the rearing pan are presumed to be 2nd instars on the 12th day from the time the eggs were laid (10 day old larvae). Most larvae will remain as 2nd instars through the 16th day (14 day old larvae). The relative size of the larval head capsule is used to confirm the instar stage. After the 16th day since the eggs were added to the rearing pan, the remaining larvae should be discarded. To maintain a supply of 2nd instar larvae for an active toxicity testing program, a rearing pan should be started every 4 days. Each pan can be expected to produce at least enough 2nd instar larvae for 3 complete acute tests.
- 10.2 Experimental Design. Ten replicates per treatment are required. The recommended test vessel is a 50 mL beaker. One 2nd instar larvae (10-14 days old) is tested in each beaker. Five or more concentrations are used for testing. A series of concentrations are selected, based on a 0.5 dilution factor i.e., each concentration is multiplied by 0.5 to obtain the next lowest concentration. The dilution factor is normally 0.5, however, other dilution factors may be used. Each study should include a control consisting of the same dilution water and conditions, but with no test compound or solvent. A solvent control is also included when appropriate. The solvent control contains a solvent concentration equal to the highest level of solvent used in a test concentration.

- 10.3 For each test concentration, pipette the appropriate amount of stock solution into one liter of dilution water and shake vigorously for one minute. Pour 40 mL of this solution into each of the ten beakers. The remaining 600 mL may be discarded or saved for water analysis (10.9).
- 10.4 Collect larvae from the rearing pan by swirling the water in the pan. This will encourage the larvae to leave their cases and swim in the open water. Pipette healthy, active larvae directly into the test beakers. Factors indicating health of larvae include relative red color intensity, size, and the degree of activity the larvae exhibit once disturbed.
- 10.5 One larva is pipetted into each beaker within 30 minutes after the compound was added. Larvae that float will not survive and should be replaced with another individual. No food is added during the test. Remove any debris from the test beakers which was inadvertently added with the larvae.
- 10.6 Test vessels are maintained at room temperature (20-22°C) without a water bath. Test solutions are not aerated during the test.
- 10.7 At the 24 hour interval, each beaker is examined. The living midges are counted by setting the beaker on a white surface illuminated by a high intensity lamp and observing whether the larva is moving. The larvae may need to be gently prodded with a pipette in order to detect any movement since midge larvae do not continuously move. Any movement detected is recorded as a live organism. The larvae are not removed from the beakers. After measurements are complete, the beakers are replaced in their previous test environment.
- 10.8 At the 48 hour interval, the beakers are again examined and the living midge larvae counted. At this time, the organisms may be sucked into a pipette to determine whether they are alive or dead. After counting, the larvae can be discarded.
- 10.9 Water Analysis. At time zero, test water used for the controls and for the highest exposure concentration should be tested for temperature (°C), dissolved oxygen, pH, alkalinity and hardness [8,9,10, 11,12,13]. At time 48 hours, the test water in the high, medium, low and control beakers should be tested for dissolved oxygen, pH alkalinity, hardness, and temperature.
- 10.10 Rangefinding Study. If there is little or no information available about the toxic level of the chemical being studied, a rangefinding study may be necessary. This preliminary test will suggest a realistic range of test concentrations to be used in the definitive study.

In the rangefinding study, 5 concentration and a control are used. The concentrations are usually spaced by a factor of 5 to 10 (e.g., 1,10,100). After 24 and 48 hours of exposure, the test chambers will be observed for mortality and/or abnormal behavioral effects. Dependent on these results, the concentration levels may be adjusted to pinpoint the LC50 in a definitive study.



## 11. CALCULATIONS

- 11.1 Test concentrations and corresponding percent mortality data derived from the definitive test are used to calculate the 48 hour median effective concentration (EC50) and its 95% confidence interval. Calculation of the EC50 is done by hand by means of the Litchfield-Wilcoxon method [14] or by a computer program designed to calculate an EC50 by means of probit analysis, moving averages, and binomial analysis [15]. The method of analysis which determines the EC50 with smallest confidence interval will be the method of analysis reported. The LC50 calculation will be based only on percent mortality and not behavior or other parameters which could be used to calculate an EC50 value.

## 12. QUALITY ASSURANCE

- 12.1 Criteria for Acceptance of a Test
- 12.1.1 Eighty percent or more of the control and solvent control survive.
  - 12.1.2 Temperature does not deviate from 22°C more than 3°C.
  - 12.1.3 Dissolved oxygen does not drop below 40 percent of saturation.
  - 12.1.4 pH does not deviate by more than one pH unit.
  - 12.1.5 At least one concentration has less than 50 percent mortality.
  - 12.1.6 At least one concentration has greater than 50 percent mortality.

## 13. REPORT

The results reported should include the following:

- 13.1 Name of test, investigator, laboratory, and date test was conducted.
- 13.2 A brief description of the toxicant including its source and lot number.
- 13.3 The source of the dilution water, its chemical characteristics, and a brief description of any pretreatment.
- 13.4 A brief description of source of Chironomus tentans, their history, experimental design and summary of methods.
- 13.5 Methods used for, and results of, all analysis of test water.

- 13.6 Methods used for, and results of, statistical analysis of data.
- 13.7 Anything unusual about the test, any deviation from the protocol, and any other relevant information.
- 13.8 Raw data. (See Appendix I).
- 13.9 Data Retention. All original raw data generated in the study will be provided to the Department of Medicine and Environmental Health staff toxicologist, and the Environmental Sciences Assessment Group GLP file. A copy of the Final Report (without raw data) will be sent to the Product Acceptability Manager, Environmental Sciences Manager, Environmental Assessment group leader and authors. Data will be retained in the MIC GLP file for ten years.

#### 14. METHOD CHANGES

- 14.1 In the event that modifications of this method are deemed necessary, a written statement of any changes and reason(s) will be provided by the study director. All agreed changes will be expressed in writing, signed and dated by the study director. The signed changes will be appended to the method and included in the final report.

#### REFERENCES

1. Bender, E.S., 1978. A Glossary of Selected Aquatic Ecological Terms. U.S. Army Special Publication ARCSL-SP-78002.
2. Post, G., 1977. Glossary of Fish Health Terms. American Fisheries Society, Fish Health Section, 48 pp.
3. Standard Operating Procedure. MIC Environmental Sciences. Cleaning Culture Tanks and Test Aquaria. Document number EAS-80-SOP-003.
4. Stephan, C.E., 1979. Proposed Standard Practice for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates, and Amphibians. Draft No. 9.
5. Stephan, C. E., 1975. Methods for Acute Toxicity Tests With Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009.
6. Special Study. MIC Environmental Sciences. Water Quality Characteristics of the Water Samples Used in the N-Building Environmental Laboratory: Part II. Report number ES-78-SS-22.
7. Standard Operating Procedure. MIC Environmental Sciences. Culturing the Midge Chironomus tentans. Document number EAS-82-SOP-44.
8. Standard Operating Procedure MIC Environmental Sciences. Temperature Measurement. Document number EAS-80-SOP-010.
9. Standard Operating Procedure MIC Environmental Sciences. Dissolved Oxygen Determination - Winkler Method. Document number EAS-80-SOP-006.

10. Standard Operating Procedure MIC Environmental Sciences. Dissolved Oxygen Determination - YSI-54ARC Operation, Maintenance, and Calibration. Document number EAS-80-SOP-011.
11. Standard Operating Procedure MIC Environmental Sciences. pH Determination. Document number EAS-80-SOP-007.
12. Standard Operating Procedure MIC Environmental Sciences. Alkalinity Measurement. Document number EAS-80-SOP-008.
13. Standard Operating Procedure MIC Environmental Sciences. Hardness Measurement. Document number EAS-80-SOP-009.
14. Litchfield, J.T. and F. Wilcoson. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour. Pharm. Exp. Ther. 96:99-113.
15. Stephan, C.E., 1978. Personal communication. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

APPENDIX I

- Raw Data Sheets -

PREPARATION OF CONCENTRATED STOCK SOLUTIONS

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Report No. \_\_\_\_\_ Date \_\_\_\_\_ Prepared by \_\_\_\_\_

Compound \_\_\_\_\_ Lot No. \_\_\_\_\_ Purity \_\_\_\_\_

Final Gross Wt. \_\_\_\_\_ Dilution Volume \_\_\_\_\_

Tare Wt. \_\_\_\_\_ Solvent \_\_\_\_\_

Net Wt. \_\_\_\_\_ Stock Conc. \_\_\_\_\_

---

MIC ENVIRONMENTAL SCIENCES REPORT NO. \_\_\_\_\_

TYPE OF TEST: \_\_\_\_\_ DATE TEST STARTED: \_\_\_\_\_ DATE TEST ENDED: \_\_\_\_\_ DIRECTED BY: \_\_\_\_\_  
 TEST SPECIES: \_\_\_\_\_ AGE: \_\_\_\_\_ CULTURE # \_\_\_\_\_ TIME TEST STARTED: \_\_\_\_\_  
 TEST MATERIAL: \_\_\_\_\_ LOT NO: \_\_\_\_\_ DATE RECEIVED: \_\_\_\_\_ PHYSICAL STATE: \_\_\_\_\_  
 FORM ADMINISTERED: \_\_\_\_\_  
 STOCK CONC: \_\_\_\_\_ TEST SOLUTION VOLUME: \_\_\_\_\_ NO. OF ANIMALS/CONC.: \_\_\_\_\_ NO. OF CONC.: \_\_\_\_\_  
 COMMENTS: \_\_\_\_\_

TEST RESULTS

TIME (HOURS)	NO. OF MORTALITIES AT EACH CONC. ( )					CHEMICAL MEASUREMENTS AT EACH CONC. ( )					OBSERVED BY:	
	24 Hr	48 Hr	72 Hr	96 Hr		TIME	CON.	DISSOLVED OXYGEN	CON.	pH		
0												
24												
48												
72												
96												
TOTAL MORTALITY												
						TIME	CON.	ALKALINITY	CON.	HARDNESS		
						0						
						24						
						48						
						72						
						96						
						TIME	CON.	TEMPERATURE				
						0						
						24						
						48						
						72						
						96						

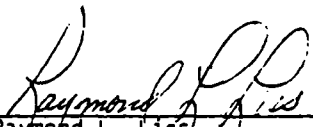
Submitted by:

Monsanto Industrial Chemicals Company  
Environmental Sciences Section - N1B  
800 North Lindbergh Boulevard  
St. Louis, Missouri 63167

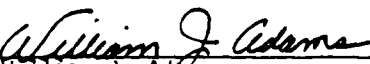
Prepared by:

  
\_\_\_\_\_  
Robert G. Mosher  
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Reviewed for  
Compliance With GLP:

  
\_\_\_\_\_  
Raymond L. Liss  
Environmental Assessment Manager

Approved by:

  
\_\_\_\_\_  
William J. Adams  
Research Group Leader

**Monsanto**

MIC ENVIRONMENTAL SCIENCES  
(CO./DIV./DEPT./LOCATION)

METHOD REPORT  
(TYPE OF REPORT)

REPORT NO.: ES-82-M-10

JOB/PROJECT NO.: 43-000-760.26-8700095

DATE: August 17, 1982

TITLE: MIC ENVIRONMENTAL ASSESSMENT METHOD FOR CONDUCTING 14-DAY PARTIAL LIFE CYCLE FLOW-THROUGH AND STATIC SEDIMENT EXPOSURE TOXICITY TESTS WITH THE MIDGE CHIRONOMUS TENTANS.

AUTHORS: R. G. Mosher, R. A. Kimerle, and W. J. Adams

ABSTRACT: Chironomus tentans larvae are exposed over a 14-day period of their life cycle (2nd to 4th instar) to 5 concentrations of a test chemical previously sorbed on sediments. Each test also includes a control (neither solvent or chemical) and a solvent control (solvent only). In one phase, clean dilution water flows through aquaria at a rate of 4 to 6 aquarium volumes per day to prevent the resolubilized chemical from reaching a significant concentration in the water. An identical test is also conducted under static conditions allowing sediment and water concentrations to achieve equilibrium. Each treatment is replicated twice using 25 C. tentans larvae per replicate yielding a total of 50 larvae per treatment. The midges are manually fed once a day during the test. After 14 days of exposure, the surviving larvae are counted and weighed. Partitioning coefficients (KP) are determined for each concentration in the static test. Effects on survival and growth are recorded for each treatment and the MATC is calculated.

AUTHORS: R. G. Mosher, R. A. Kimerle and W. J. Adams  
TITLE: MIC ENVIRONMENTAL ASSESSMENT METHOD FOR CONDUCTING 14-DAY CHRONIC FLOW-THROUGH AND STATIC TOXICITY TESTS WITH THE MIDGE CHIRONOMUS TENTANS.



MIC ENVIRONMENTAL ASSESSMENT METHOD FOR CONDUCTING  
14-DAY CHRONIC FLOW-THROUGH AND STATIC TOXICITY TESTS  
WITH THE MIDGE CHIRONOMUS TENTANS

1. SCOPE

- 1.1 This method describes a procedure to determine the concentrations of a sediment sorbed chemical which produce chronic effects in a 14-day period in the life cycle of the midge Chironomus tentans, a benthic invertebrate.
- 1.2 One phase of this method involves a flow-through system and another a static system. The parameters used to determine chronic toxicity are survival and growth. The sediment-water partitioning coefficient (Kp) may also be determined.
- 1.3 This method is designed for those chemicals which readily partition to the sediment.
- 1.4 Careful consideration should be given to those chemicals which might biodegrade, volatilize, oxidize, or photolyze during the test period. If the chemical concentration on the sediment changes significantly within the duration of the test, this method may not be applicable.
- 1.5 This method can probably be used to conduct chronic tests with other species of the midge family, Chironomidae. However, some modifications may be necessary.

2. APPLICABLE DOCUMENTS

2.1 ASTM Standards

D511	Calcium and Magnesium
D512	Chloride
D516	Sulfate
D857	Aluminum
D888	Oxygen, Dissolved in Water
D1067	Acidity and Alkalinity
D1125	Conductivity
D1126	Hardness
D1129	Definitions
D1179	Fluoride
D1193	Reagent Water
D1252	Oxygen Demand, Chemical
D1253	Chlorine Residual, Water
D1293	pH
D1426	Ammonia

D1428	Potassium and Sodium
D1888	Solids, Particulate and Dissolved in Water
D2576	Metals by Atomic Absorption
D2579	Total Organic Carbon
D2972	Arsenic
D3082	Boron
D3086	Pesticides
D3223	Mercury
E380	Metric Practice Guide

### 3. SUMMARY

- 3.1 Chironomus tentans larvae are exposed over a 14-day period of their life cycle (2nd to 4th instar) to 5 concentrations of a test chemical previously sorbed on sediments. Each test also includes a control (neither solvent or chemical) and a solvent control (solvent only). In one phase, clean dilution water flows through aquaria at a rate of 4 to 6 aquarium volumes per day to prevent the resolubilized chemical from reaching a significant concentration in the water. An identical test is also conducted under static conditions allowing sediment and water concentrations to achieve equilibrium. Each treatment is replicated twice using 25 C. tentans larvae per replicate yielding a total of 50 larvae per treatment. The midges are fed once a day during the test. After 14 days of exposure, the surviving larvae are counted and weighed. Partitioning coefficients ( $K_p$ ) are determined for each concentration in the static test. Effects are calculated for each treatment level.

### 4. SIGNIFICANCE

- 4.1 The midge, Chironomus tentans, along with other members of the Family Chironomidae make up a significant portion of macroinvertebrate populations in many freshwater ecosystems. They serve as an important source of food for young and adult fish along with other aquatic animals. A major change in the availability of C. tentans as a food organism could have serious adverse ecological effects on the entire aquatic system.
- 4.2 The proposed chronic toxicity practice using C. tentans is designed to assess the effects of a toxicant on the survival and growth of laboratory populations of a representative macroinvertebrate. The results of a given study can be used as part of a hazard assessment program to determine the potential hazard associated with the presence of a chemical in the environment. Water quality criteria for a given chemical or physical parameter may also be derived from the study results.

### 5. DEFINITION OF TERMS

- 5.1 Toxicity - Quality, state, or degree of harmful effect resulting from alteration of an environmental factor.

- 5.2 Toxicity Test - An experimental study designed to measure the degree of harmful effects resulting from an alteration in an environmental factor.
- 5.3 Toxic Agent - A substance which kills or impairs health through its chemical or physical action.
- 5.4 Toxicant - A substance (a poison) when taken into or formed in the body, kills or impairs health.
- 5.5 Midge Partial Life Cycle Toxicity Test - An experimental study of the survival and reproduction of the midge Chironomus tentans through a major portion of their life cycle.
- 5.6 Test Concentration - The dose or quantity of toxicant sorbed to the sediments to which the C. tentans are exposed for 14 days.
- 5.7 Treatment(s) - Refers to the groups of C. tentans which are exposed to the toxicant as opposed to the controls.
- 5.8 Stock Solution - A concentrated mixture of the toxicant and dilution water or carrier solvent which is mixed with dilution water to prepare a test solution.
- 5.9 Water Delivery System - The system which delivers equal amounts of clean dilution water to the test chambers.
- 5.10 For definitions of other terms in this practice, refer to ASTM Definitions D1129, Terms Relating to Water (1). For an explanation of units and symbols, refer to ASTM Standard E380, Metric Practice Guide (4).

## 6. SAFETY PRECAUTIONS

- 6.1 Many substances may adversely affect human beings if adequate precautions are not taken. Therefore, contact with all toxicants and test solutions should be minimized, and special precautions, such as covering test chambers and increasing ventilation, should be taken with volatile toxicants. Information on toxicity to humans and recommended handling procedures (5) should be studied before tests are begun with any toxicant.
- 6.2 Although disposal of stock solutions, test solutions, and test organisms poses no special problems in most cases, health and safety precautions should be considered before beginning a test. Removal or degradation of toxicant before disposal of stock and test solutions is sometimes desirable.
- 6.3 Rinsing with acetone and other volatile solvents should be performed only in well-ventilated areas.
- 6.4 Acid and hypochlorite solutions should not be mixed because hazardous fumes may be produced.

7. APPARATUS

- 7.1 Facilities. The facilities should include a constant-temperature area or recirculating water bath for culture tanks and test chambers. A dilution-water tank may be used to prepare reconstituted water. The tank may be elevated so dilution water can flow by gravity into culture tanks and test chambers, and should be equipped for temperature control and aeration. Strainers and air traps should be included in the water supply system. Air used for aeration must be free of oil and fumes; filters to remove oil and water are desirable. During culture and testing, test organisms should be shielded from disturbances. The test facility must be well ventilated and free of fumes. A 16-hour light and 8-hour dark photo period should be provided. A 15- to 30-minute transition period between light and dark may be desirable. Light intensity should be 400-800 lux (37-74 foot candles) at the surface of test solutions and provided by wide-spectrum (Color Rendering Index 90) fluorescent lamps.
- 7.2 Construction materials. Construction materials and commercially purchased equipment that may contact stock solutions, test solutions or any water into which the midges will be placed should not contain any substance that can be significantly leached or dissolved by aqueous solutions. In addition, materials and equipment that contact stock solutions or test solutions should be chosen to minimize sorption of toxicants from water. To minimize leaching, dissolution and sorption, glass, #316 stainless steel, high density polyethylene and perfluorocarbon plastics must be used whenever possible.
- 7.3 Test chambers. Aquaria are constructed of glass and silicone rubber with a volume of 3 L. Test chambers measure 20.5 x 12.5 x 14.5 cm with a 12.5 x 4.5 cm piece of fine mesh stainless steel screen positioned on the upper end of one side. This overflow screen prevents the escape of larvae and maintains a test solution volume of 2 L. In flow-through systems, glass box (14 x 10 x 5 cm) flow splitters divide the test solutions from the diluter system and delivery 250 mL to each replicate. Each flow splitter has two 5/8" holes in the bottom which are fitted with #3 silicone stoppers. A small diameter glass tube is inserted through the stoppers to minimize turbulence in the test chambers.
- 7.4 Cleaning. Test chambers and equipment used to prepare and store dilution water, stock solutions, and test solutions must be cleaned each time before use according to EAS-80-SOP-003 (6).

8. DILUTION WATER

- 8.1 General requirements. An adequate supply of a freshwater that is acceptable for culturing and testing Chironomus tentans must be available. Flow-through tests will not need aeration under normal circumstances. Static tests are gently aerated through a pipette held under the water surface. The specifications listed in 8.2

and 8.3 were selected mainly to help insure that the dilution water is acceptable for culturing and testing C. tentans.

- 8.2 Natural freshwater dilution water. Natural freshwater dilution water should be uncontaminated and of constant quality and should meet the following specifications:

Particulate matter	<20 mg/Liter
TOC or	< 2 mg/liter
COD	< 5 mg/liter
Un-ionized ammonia	<20 ug/Liter
Residual chlorine	< 3 ug/liter
Total organophosphorus pesticides	<50 ng/liter
Total organochlorine pesticides plus PCB's (or organic chlorine)	<50 ng/liter <25 ng/liter
Hardness (mg/L CaCO <sub>3</sub> )	>100 mg/liter
pH	7.0-8.2

A natural dilution water is considered to be of constant quality if the monthly ranges of the hardness, alkalinity, and specific conductance are less than 10 percent of their respective averages and if the monthly range of pH is less than 0.4 unit. Natural dilution waters should be obtained from an uncontaminated well or spring if possible or from a surface water source. Only as a last resort should a dechlorinated water be used. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chlorine or chloramines.

- 8.3 Reconstituted dilution water. Reconstituted water is prepared by adding specified amounts (Table 1) of reagent-grade (7) chemicals to water which meets the following specifications:

Un-ionized ammonia	<20 ug/liter
Aluminum, arsenic, chromium, cobalt copper, iron, lead, nickel, zinc	< 1 ug/liter each
Residual chlorine	< 3 ug/liter
Cadmium, mercury, silver	<100 ng/liter each
Total organophosphorus pesticides	<50 ng/liter
Total organochlorine pesticides plus PCBs (or organic chlorine)	<50 ng/liter <25 ng/liter
Hardness (mg/L CaCO <sub>3</sub> )	>100 mg/L
pH	7.0-8.2

Glass-distilled water and carbon-filtered deionized water are generally acceptable. Conductivity, pH and hardness must be measured on each batch from which reconstituted water is to be prepared. The other characteristics must be measured at least twice a year and whenever significant changes in these characteristics are expected. If the water is prepared from a surface water, TOC or COD must be measured on each batch.

8.4 St. Peter's well water is used in the Environmental Sciences Laboratories at Monsanto. This water meets EPA & ASTM's standards of quality for natural freshwater dilution water and has been used successfully to culture and test various aquatic organisms for several years.

## 9. TOXICANT

- 9.1 The toxicant should be reagent grade or better, except for tests on formulations or commercial products. If the identity and concentration of major ingredients and major impurities are not known, they should be determined. The toxicant should be added to the sediments without the use of solvents if possible. If a solvent is necessary, it must be one which can be driven off (i.e., evaporated) leaving only the test chemical on the sediments.
- 9.2 The stability of the toxicant in the stock solution should be verified by chemical analyses. Stock solution sub samples should be analyzed immediately after the stock has aged the maximum length of time it would be in use in the test.

## 10. SEDIMENTS

- 10.1 The sediments used in the Environmental Sciences Laboratories are obtained from local undisturbed agricultural soils. These soils can be used as found or mixed with a high organic carbon (OC) soil such as a composted manure. The sediment chosen for use should be characterized and at least the following should be known: pH, % organic carbon, % sand, % silt, % clay and % water holding capacity.
- 10.2 Sediments are prepared for use by heating at 100°C for 12 hours and sieving through a No. 25 U. S. Standard Sieve (710 micron opening). All characterizations should be made after the soil has been heated and sieved.

## 11. TEST ORGANISMS

- 11.1 Chironomus tentans is the recommended test species because of its relatively large size, ease of culture and handling and habit of burrowing into sediments to build a case retreat. This method could easily be adapted to other species of midges with similar habits.
- 11.2 The identity of organisms obtained from laboratories and commercial sources should be verified regardless of any information that comes with the organisms, since such information is not always accurate.
- 11.3 Handling. Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible, so that the organisms are not unnecessarily stressed. Organisms should be introduced beneath the air/water interface. Any organisms that touch dry surfaces or are dropped or injured during handling should be discarded.

AD-A170 870

BIOASSESSMENT METHODOLOGIES FOR THE REGULATORY TESTING  
OF FRESHWATER DRED. (U) ARMY ENGINEER WATERWAYS  
EXPERIMENT STATION VICKSBURG MS ENVIR.

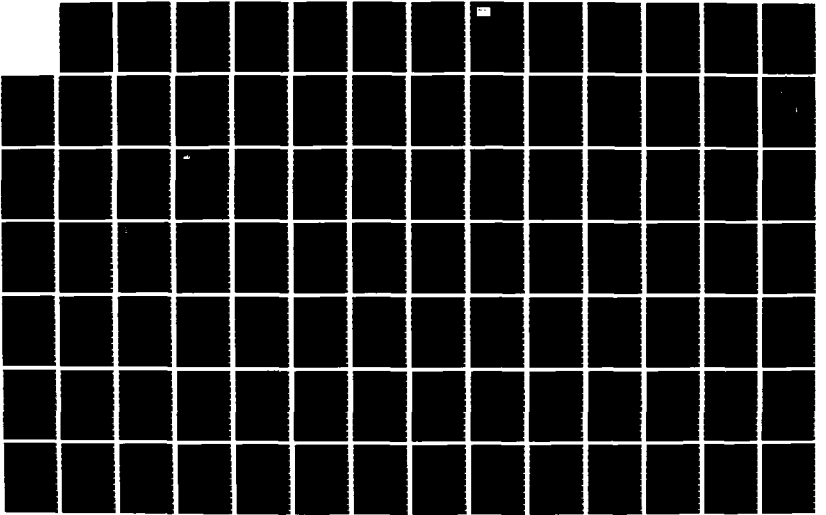
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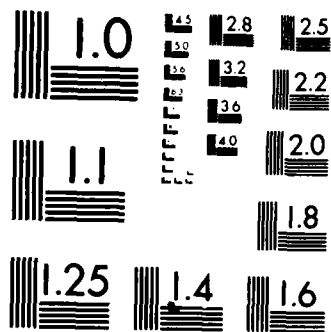
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MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A



- 11.4 Food preparation. Midge food is prepared according to instructions given in EAS-82-SOP-44 (8).

12. PROCEDURE

- 12.1 Preliminary preparations. Twelve to 16 days before an acute test is begun, 3 freshly laid *Chironomus tentans* egg masses are placed in a clean 20x40 cm glass or enameled rearing pan filled with well water to a depth of 3 cm. No substrate is added to the pan. At 20°C, larvae will begin to appear in 48 hours. Food (Tetra Conditioning Food Vegetable Diet suspension) is then added at the rate of .5 mL per day (8). Fresh water is added as needed to make up for that lost to evaporation. The larvae in the rearing pan are presumed to be 2nd instars on the 12th day from the time the eggs were laid (10 day old larvae). Most larvae will remain as 2nd instars through the 16th day (14 day old larvae). The relative size of the larval head capsule is used to confirm the instar stage. After the 16th day since the eggs were added to the rearing pan, the remaining larvae should be discarded. To maintain a supply of 2nd instar larvae for an active toxicity testing program, a rearing pan should be started every 4 days. Each pan can be expected to produce at least enough 2nd instar larvae for a complete 14-day chronic test. However, if static and flow-through tests are to be run concurrently, two rearing pans of suitable age should be available.
- 12.2 Experimental design. A minimum of 5 treatment concentrations, one control and one solvent control, each with two replicates, are required for both static and flow-through tests. Prepared soil is added to each test chamber. The recommended amount is 100 g, but other amounts may be used as test material properties dictate. Flow-through test chambers are arranged under a diluter system. Static aquaria can be located in any area having aeration facilities which meet the requirements of 7.1.
- 12.3 The delivery system of the dilution water can be one of several designs. The system should satisfy the two following functions: a) must be capable of delivering four to six volume exchanges of dilution water per day; b) must be capable of delivering equal amounts of water to two replicates each of 7 concentrations. Schematics for one delivery system are shown in Figure 1.
- 12.4 At least one control and five toxicant concentrations in a geometric series with a certain dilution factor should normally be used. More treatments may be desirable to insure the acceptability of the test. If information is available from a previous test, it might be determined with fewer than six treatments. However, as the number of toxicant concentrations is decreased, the risk of all concentrations being either too high or too low is greatly increased. A chronic toxicity test with *C. tentans* should not be started until after the 48-hour LC50 has been determined, both with the organisms unfed and in the presence of 15 mg/L food (9).

- 12.5 In order to determine the lowest test concentration which has a significant effect upon the survival and reproduction of the midges, it is necessary that: a) at least one test concentration has an effect on survival or reproduction as compared to the control; b) toxicant effects on all lower concentrations are not significantly different than the controls; and c) all higher concentrations do significantly affect survival or reproduction.
- 12.6 Test chemicals are spiked onto dry prepared soils already in test chambers. The solution of test chemical is homogenized into the dry soil with excess amounts of a volatile solvent such as acetone. The aquaria are then placed under a ventilated hood. To facilitate measurement of test chemical concentration, it is recommended that radiolabeled test material be used. At least 3 replicates of sediment weighing 0.1 g each are analyzed from each spiked treatment replicate.
- 12.7 The water delivery system should be started several days before the study begins to remove any excess of the chemical desorbing from the sediment. Concentrations of the chemical in the water must be monitored before the test begins. The organisms are added when the concentrations in the water are below any possible effect level. Thereafter, water concentrations are monitored on days 0, 1, 4, 7, 10 and 14. Midges can be added to static test chambers 24 hours after 2 liters of dilution water are added. Water concentrations are monitored as above.
- 12.8 Any floating debris should be skimmed from the test chambers before midge larvae are added. This is accomplished with a piece of fine nylon screen. When high OC sediments are used, this debris can be considerable due to woody fragments in the soil. If more than 0.1 g of floating debris is removed, an analysis should be performed to determine the amount of chemical removed from the system.
- 12.9 The test begins when the midge larvae are added to the test chambers. It is recommended that flow-through and static tests be started on different days to assure that sufficient time is available to complete all tasks. Care is taken to introduce the larvae below the air-water interface. Test chambers should be inspected several hours after larvae are introduced to insure that no larvae are trapped on the surface tension of the water. These "floaters" do not survive well and should be replaced with healthy larvae.
- 12.10 Dissolved oxygen concentration. Test solutions in flow-through aquaria need not be aerated under normal conditions. Static test aquaria should be gently aerated starting one day after the larvae have been introduced. This aeration must not disturb the sediment.
- 12.11 Feeding. Larvae are fed by hand once a day using "Tetra Conditioning Food Vegetable Diet: suspension (8)". A feeding consists of approximately 50 mg dry solids administered in a 0.5 mL suspension of food and water to each aquarium. Excess food should not be allowed to collect on the sediment and permit the growth of fungus. If this occurs, the feeding rate should be reduced.

- 12.12 Temperature. C. tentans will thrive under the normal range of room temperatures. However, for testing purposes, temperature should not deviate from  $22^{\circ} \pm 3^{\circ}\text{C}$ . Temperature is measured daily (10).
- 12.13 Water Quality Analysis
- 12.13.1 The laboratory dilution water should be characterized. The results of analytical measurements on the dilution water should accompany the final report. Typical measurements include those listed in Section 2.1 and 8.2. Semi-annual measurement of these parameters is adequate if experience indicates the dilution water characteristics are constant.
- 12.13.2 The hardness, alkalinity, pH and conductivity of the test solution are measured before larvae are added, on day 7 and on day 14. These measurements must be taken in compliance with the appropriate SOP (11,12,13).
- 12.13.3 Dissolved oxygen is measured on days 0, 1, 4, 7, 10, and 14. To minimize disturbance to the test system, a dissolved oxygen meter is used (14). D.O. levels should not be allowed to fall below 50% of saturation.
- 12.14 Termination of tests. At the end of the 14-day test period, the test solution is carefully decanted from the test chambers. The larvae are then picked from the sediments, cleaned of foreign matter, and placed in an oven dried cellulose cone of known weight. The midge bodies from each replicate aquaria are dried in the cone for 2 hours at  $100^{\circ}\text{C}$ . The cones are then weighed to determine the dry weight of the midges from each aquaria.
- 12.15 Measurement of interstitial water and sediment concentrations. The mud remaining in the test chambers is mixed well with a spatula until it appears uniform in consistency. A high strength glass centrifuge tube (Corex , 25 mL) is then filled 3/4 full with the wet sediment for each of the aquaria. The tubes are then centrifuged at 12,000 rpm for 20 minutes. The decantant and sediment pellet are then analyzed for test chemical concentration.
- 12.16 Midge bodies may be analyzed for test chemical concentrations and bioconcentration factor may be determined.
- 12.17 The analytical method used to measure the concentration of toxicant in the sediments and test solutions must be validated before the beginning of the test.

13. CRITERIA FOR ACCEPTING A TEST

- 13.1 Test organisms must be cultured in compliance with the appropriate SOP.
- 13.2 The control and solvent control must have eighty percent total survival.
- 13.3 At least one toxicant concentration must not have a statistically significant effect on the biological factors measured. There must also be one concentration that does have an effect.
- 13.4 Analytical measurements of test solutions must not be extremely variable.
- 13.5 Sediments must have greater than 50% of the original nominal concentration at the conclusion of the test.
- 13.6 Dissolved oxygen concentration must be maintained at 50% or better of saturation.
- 13.7 pH Must not change more than  $\pm 1$  unit.
- 13.8 Temperature must not average 3°C higher or lower than 22°C or cannot deviate from 22°C by 5°C at any given time.

14. CALCULATIONS

- 14.1 Statistical evaluations which are often used to evaluate the effects of a given toxicant on the survival and growth of C. tentans include two way analysis of variance and Dunnett's T test (15).
- 14.2 The analysis of variance test will provide information on the effects of the toxicant among the replicates. Dunnett's T test is used to determine whether one specific treatment level is significantly different than the control.
- 14.3 Partition coefficients are calculated on the basis that

$$K_P = \frac{\text{concentration sediment}}{\text{concentration water}}$$

Sediment and water concentrations must have compatible units (i.e., .1 g sediment and .1 mL water) (16).

- 14.4 Bioconcentration Factors (BCF) are determined by

$$\frac{\text{concentration organism}}{\text{concentration sediment}} \quad \text{or}$$

$$\frac{\text{concentration organism}}{\text{concentration water (average exposure)}} \quad (17)$$


REFERENCES

1. Annual Book of ASTM Standards, Part 31, Philadelphia, Pa., American Society of Testing & Materials, 1979.
2. Bender, E. S. 1978. A Glossary of Selected Aquatic Ecological Terms. U.S. Army Special Publication ARCSL-SP-78002.
3. Post, G. 1977. Glossary of Fish Health Terms. American Fish Soc. Fish Health Sec., 48 pp.
4. Annual Book of ASTM Standards, Part 41, Philadelphia, Pa., American Society for Testing & Materials, 1976.
5. For example, see: Manufacturing Chemists Association, 1975. Laboratory Waste and Disposal Manual. Washington, D.C. 176. pp.
6. EAS-80-SOP-003. Cleaning Culture Tanks and Test Aquaria. 1p.
7. For example, see: U.S. Environmental Protection Agency. 1974. Methods for Chemical Analysis of Water and Wastes. Methods Development and Quality Assurance Research Laboratory. Cincinnati. 298 pp; J.D.H. Strickland and T. R. Parsons. 1968. A Practical Handbook of Seawater Analysis Fish. Res. Bd. Canada. Bulletin 167, Ottawa; National Handbook of Recommended Methods for Water-Data Acquisition. 1977. U.S. Department of the Interior; American Public Health Association. Amer. Waterworks Assoc., Water Pollution Control Federation. 1975.
8. Standard Operating Procedure MIC Environmental Sciences. Procedure for Culturing the Midge Chironomus tentans. EAS-82-SOP-49.
9. Mosher, R. G. and W. J. Adams. 1982. Method for Conducting Acute Toxicity Tests With the Midge Chironomus tentans Report No. ES-82-M-7.
10. EAS-80-SOP-007. pH Determination.
11. EAS-80-SOP-008. Alkalinity Measurement.
12. EAS-80-SOP-009. Hardness Determination in Test and Culture Waters.
13. EAS-80-SOP-010. Temperature Measurement.
14. EAS-80-SOP-011. Dissolved Oxygen Determination YSI-54 ARC Meter and Probe Method.
15. Snedecor, G.W. and W. G. Cochran. 1976. Statistical Methods, Iowa State University Press, Ames, Iowa. 593 pp.
16. Michael, P.R. and R. G. Kaley. 1979. A Method for the Determination of Soil/Water Partition Coefficients. Report No. ES-79-M-19.
17. Heidolph B. B. 1980. Method for Conducting Bioconcentration Tests With Fishes. Report No. ES-80-M-33.

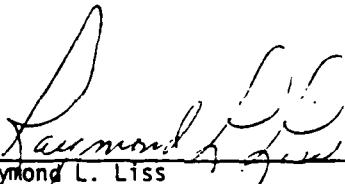
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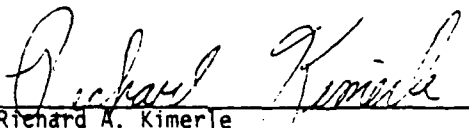
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MIC ENVIRONMENTAL SCIENCES  
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METHOD REPORT  
(TYPE OF REPORT)

REPORT NO.: ES-82-M-11

JOB/PROJECT NO.: 43-000-760.26-8700095

DATE: August 24, 1982

TITLE: MIC ENVIRONMENTAL ASSESSMENT METHOD FOR CONDUCTING  
14-DAY WATER EXPOSURE PARTIAL LIFE CYCLE TOXICITY  
TESTS WITH THE MIDGE CHIRONOMUS TENTANS

AUTHORS: R. G. Mosher, R. A. Kimerle, and W. J. Adams

ABSTRACT: Second instar midge larvae are exposed to a toxicant in an intermittent flow system designed to exchange the total volume of test water and toxicant three to six times per day. At least five concentrations, a control, and a solvent control (if appropriate) are recommended. Controls are treated the same as the treatments except they are not exposed to the toxicant. Each test concentration is replicated. At the end of the 14-day test period, larvae are counted and weighed. Control midges are expected to reach fourth instar (the stage preceeding pupation). Toxicity effects are determined from survival and growth measurements.

MIC ENVIRONMENTAL ASSESSMENT METHOD FOR CONDUCTING  
14-DAY WATER EXPOSURE CHRONIC TOXICITY TESTS WITH THE MIDGE CHIRONOMUS TENTANS

1. SCOPE

- 1.1 This method describes a procedure for determining the chronic toxicity of chemicals in water to a representative benthic invertebrate, the midge Chironomus tentans (Diptera: chironomidae).
- 1.2 This procedure is applicable to most toxicants. However, materials that readily partition to sediments, i.e., those with a high sediment partition coefficient (Koc), may require special consideration. Because of their strong tendency to sorb to particles (especially organic carbon), it is recommended that materials with a high Koc value also be studied in tests employing a sediment or food exposure route.

2. APPLICABLE DOCUMENTS

2.1 ASTM Standards

D511	Calcium and Magnesium <sup>a</sup>
D512	Chloride <sup>a</sup>
D516	Sulfate <sup>a</sup>
D857	Aluminum <sup>a</sup>
D888	Oxygen, Dissolved in Water <sup>a</sup>
D1067	Acidity and Alkalinity <sup>a</sup>
D1125	Conductivity <sup>a</sup>
D1126	Hardness <sup>a</sup>
D1129	Definitions <sup>a</sup>
D1179	Fluoride <sup>a</sup>
D1193	Reagent Water <sup>a</sup>
D1252	Oxygen Demand, Chemical <sup>a</sup>
D1253	Residual Chlorine in Water <sup>a</sup>
D1293	pH <sup>a</sup>
D1426	Ammonia <sup>a</sup>
D1428	Potassium and Sodium <sup>a</sup>
D1888	Solids, Particulate and Dissolved in Water <sup>a</sup>
D2576	Metals by Atomic Absorption <sup>a</sup>
D2579	Total Organic Carbon <sup>a</sup>
D2972	Arsenic <sup>a</sup>
D3082	Boron <sup>a</sup>
D3086	Pesticides <sup>a</sup>
D3223	Mercury <sup>a</sup>

<sup>a</sup>1976 Annual Book of ASTM Standards, Part 31



### 3. SUMMARY

- 3.1 Second instar midge larvae are exposed to a toxicant in an intermittent flow system designed to exchange the total volume of test water and toxicant three to six times per day. At least five concentrations, a control (receiving no chemical) and a solvent control (for those chemicals requiring a solvent) are recommended. Controls are treated the same as the treatments except they are not exposed to the toxicant. Each test concentration is replicated. At the end of the 14-day test period, larvae are counted and weighed. Control midges are expected to reach 4th instar (the stage preceding pupation). Toxicity effects are determined from survival and growth measurements.

### 4. SIGNIFICANCE

- 4.1 The larvae of the midge Chironomus tentans are aquatic organisms that construct tubes of sediment and detritus within the sediment of a variety of freshwater habitats. They live in these cases until they emerge as adult flying insects. The benthic life and sediment handling characteristics of the larval stages of this organisms make it a useful model for evaluating the toxicity of chemicals. Also, its relatively large size and ease of culture make it amenable to laboratory toxicological investigations.
- 4.2 This procedure is designed to assess the effect of toxicants in aqueous solution on the survival and growth of C. tentans. The results of a given study can be used as a part of a safety assessment program.

### 5. DEFINITION OF TERMS

- 5.1 Toxicity - Quality, state, or degree of harmful effects resulting from and exposure to a toxicant (1).
- 5.2 Toxicity Test - An experimental study designed to measure the degree of harmful effects resulting from an exposure to a toxicant.
- 5.3 Toxicant - A substance (a poison) which when taken into or formed in the body, kills or impairs health (2).
- 5.4 Toxic Agent - A substance which kills or impairs health through its chemical or physical action.
- 5.5 Midge Partial Life Cycle Toxicity Test - An experimental study of the survival and growth of the midge Chironomus tentans through a major portion of their life cycle.
- 5.6 Diluter System - A system which mixes dilution water and toxicant in specific predetermined ratios on a continuous or intermittent basis and delivers the test solutions and control water to the test vessels.
- 5.7 Test Concentration - The dose or quantity of toxicant placed in solution in the dilution water to which the midge are exposed.

- 5.8 Treatment(s) - Refers to the midge which are exposed to the toxicant as opposed to the controls.
- 5.9 Test Solutions - A mixture of the toxicant and the dilution water in which the midge reside during the study.
- 5.10 Stock Solution - A concentrated mixture of the toxicant and dilution water or carrier solvent, which is mixed with dilution water to prepare a test solution.
- 5.11 MATC - Maximum Acceptable Toxicant Concentration. The MATC is the highest toxicant concentration that does not produce a statistically significant effect on the biological parameters measured.
6. APPARATUS
- 6.1 Facilities. During culturing and testing, disturbance of organisms should be minimized. A 16-hour light and 8 hour dark photoperiod is provided. Light intensity is provided by wide spectrum (Color Rendering Index 90) fluorescent lamps and should be 400-800 lux (34-74 foot candles).
- 6.2 Exposure Apparatus. Test chambers consist of 3 liter, all glass aquaria measuring 12.5x20.5x14.5 cm. Silicone rubber sealant is used to cement the glass together. The upper 6.5 cm of one end of the aquaria consists of a piece of fine mesh stainless steel screen. This allows water to drain from the aquaria while retaining the larvae. The test chambers hold 2 liters of test solution. Glass box (14x10x5 cm) flow splitters divide the test solutions from the diluter system between the replicates. Each flow splitter has two 5/8" holes in the bottom which are fitted with #3 silicone stoppers. A 1/8" diameter glass tube is inserted through each stopper. This allows for the delivery of the toxicant and avoids excessive turbulence in the beakers.
- 6.3 Cleaning. Test chambers and equipment used to prepare and store dilution water, stock solutions, and test solutions must be cleaned before use, see SOP #EAS-80-SOP-003 (3).
- 6.4 Toxicant Delivery System.
- 6.4.1 The diluter system used is an intermittent flow solenoid diluter system described by Adams et al. (Figure 1) (4).
- 6.4.2 The toxicant-delivery system is calibrated before each test. This includes measuring the flow rate and volume to each test chamber and the concentration of toxicant in each test chamber. Flow rates through test chambers do not vary by more than 10% from any one test chamber to any other or from one time to another within a test. Operation of the toxicant delivery system will be checked daily (except on weekends) for normal operation throughout the test.

7. REAGENTS AND MATERIALS

7.1 Dilution Water

7.1.1 Commonly used dilution waters are from wells or surface waters and should be uncontaminated, of constant quality, and meet the following specifications:

Particulate matter	<20 mg/liter
TOC or COD	< 5 mg/liter
Un-ionized ammonia	<20 µg/liter
Residual chlorine	< 3 µg/liter
Total organophosphorus pesticides	<50 ng/liter
Total organochlorine pesticides plus	<50 ng/liter
PCB's or organic chlorine	<25 ng/liter
Hardness (mg/L CaCO <sub>2</sub> )	>100 mg/liter
pH	7.0-8.2
Boron, fluoride	<100 µg/liter each
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc	< 1 µg/liter each
Cadmium, mercury, silver	<100 ng/liter each

7.1.2 A natural dilution water is considered to be of constant quality if monthly ranges of hardness, alkalinity, and specific conductance are less than 10 percent of their respective averages and if the monthly range of pH is less than 0.4 units. Only as a last resort will dechlorinated city water be used. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chloride, or chloramines.

7.1.3 The recommended dilution water is St. Peter's well water. This water has been used for the past several years in the Environmental Sciences aquatic laboratory. The history of the water is well documented and it has been shown to be of high quality. Well water is the water of choice because it so closely approximates natural surface waters, is free of chemical contamination, and is used in time independent and chronic toxicity tests with acceptable survival, growth, and reproduction (5).

7.1.4 Reconstituted Dilution Water. Reconstituted water is prepared by adding specified amounts of chemicals to high quality distilled or ionized water. Reconstituted water should only be used when measuring the effects of chemical parameters (pH, hardness, etc.) on the toxicity of a toxicant or for inter-laboratory comparative toxicity tests. Reconstituted water can be prepared as described in the reference (5).

7.2 Toxicant. The major components of the toxicant should be known. The toxicant should be added to the dilution water without the use of solvents or other chemicals, if possible. If carriers other than water are necessary, the amount used must be kept to a minimum, preferably less than or equal to 0.5 mL solvent/liter test water.

8. PRECAUTIONS

- 8.1 Some substances can adversely affect human beings if adequate precautions are not taken. Therefore, contact with all toxicants and test solutions should be minimized. Information on toxicity to humans and recommended handling procedures should be studied before tests are begun with any toxicant.

9. TEST ORGANISM

- 9.1 Because of its ease of culture and relatively large size, Chironomus tentans is the recommended test organism.
- 9.2 Transfer of midges from rearing pan to test chamber is accomplished with a Pasteur pipette with a polished opening. To avoid undue stress and injury to the larvae, handling should be gentle and as brief as possible.
- 9.3 Second instar larvae used in tests are derived from eggs obtained from C. tentans cultures maintained according to SOP #EAS-82-SOP-044 (6).

10. PROCEDURE

- 10.1 Preliminary Preparations. Twelve to 16 days before an acute test is begun, 3 freshly laid Chironomus tentans egg masses are placed in a clean 20x40 cm glass or enameled rearing pan filled with well water to a depth of 3 cm. No substrate is added to the pan. At 30°C, larvae will begin to appear in 48 hours. Food (Tetra Conditioning Food Vegetable Diet suspension) is then added at the rate of .5 mL per day (6). Fresh water is added as needed to make up for that lost to evaporation. The larvae in the rearing pan are presumed to be 2nd instars on the 12th day from the time the eggs were laid (10 day old larvae). Most larvae will remain as 2nd instars through the 16th day (14 day old larvae). The relative size of the larval head capsule is used to confirm the instar stage. After the 16th day since the eggs were added to the rearing pan, the remaining larvae should be discarded. To maintain a supply of 2nd instar larvae for an active toxicity testing program, a rearing pan should be started every 4 days. Each pan can be expected to produce at least enough 2nd instar larvae for a complete test.
- 10.2 Experimental Design. At least 5 test solution concentrations consisting of two replicates each are required. A solvent control and a control, each with two replicates, are also needed. The solvent control contains the highest amount of solvent used in any treatment and the control contains only dilution water. The concentrations used in the treatments are selected through information obtained from acute toxicity test data (7) and follow a 0.5 dilution factor. Radiolabeled toxicant material may be used to facilitate concentration measurements.

- 10.3 Substrate. For optimal growth and survival, *C. tentans* larvae require a substrate suitable for use in the construction of a case. Abnormal behavior patterns, such as cannibalism, may result if the larvae are deprived of sufficient substrate. Soil that has been heated in a muffle furnace (500°C for 4 hours) serves this purpose as it provides *C. tentans* larvae with substrate but contributes virtually no organic carbon to the test system. Any naturally low OC soil is prepared for muffling by first being dried at 100°C and then sieved through a No. 25 U.S. Standard Sieve (700 micron opening). Each test chamber receives 100g of the muffled soil.
- 10.4 Twenty-five larvae are carefully added to each filled test chamber. Larvae are introduced beneath the air/water interface by pipette. After several hours, each aquarium should be inspected for midges trapped on the surface tension of the water. These "floaters" do not survive well and should be replaced with a fresh larva.
- 10.5 Temperature. The ideal range for testing is  $22 \pm 2^\circ\text{C}$ . Any anticipated variance from this range should be countered with a water bath of suitable temperature. Temperature measurements are made daily (8).
- 10.6 Dissolved oxygen. Aeration should not be necessary if the diluter system is functioning properly. Dissolved oxygen measurements are made at the beginning of the study and twice weekly thereafter. If low D.O. is suspected, a measurement should be taken immediately. A D.O. meter is used to avoid removing larger quantities of water (9).
- 10.7 Feeding. Feeding is done by hand once a day. The food consists of a suspension of "Tetra-Min Conditioning Food Vegetable Diet" available at most pet stores (13). The recommended daily dose of food suspension contains approximately 50 mg of dry solids which is delivered in 0.5 mL. If food collects on the substrate, feeding may be suspended for one or more days.
- 10.8 Alkalinity, hardness, and pH are measured at the beginning of the test and weekly thereafter in the control, low, medium, and high concentrations (10,11,12).
- 10.9 Water samples for toxicant analysis are taken on days 0, 1, 4, 7, 10, and 14. If radiolabeled material is used, up to a 3 mL water sample may be counted, with two replicates taken from each of the 14 aquaria.
- 10.10 The study is ended 14 days after the larvae are introduced into the system. Each aquaria is carefully searched for larvae which are gently rinsed and removed. When all larvae have been found, they are placed in a cellulose cone of known weight and dried in an oven at 100°C for 2 hours. Larvae are then weighed together in the cone.

## 11. CALCULATIONS

- 11.1 Data on percent survival and growth (average weight) are statistically analyzed. Statistical evaluation of the results compares the control to all other treatments including the solvent control. The measured parameters from duplicate test chambers are subjected to analysis of variance (ANOVA) and treatment means compared using Dunnett's t-test (13).
- 11.2 Bioaccumulation levels are determined by the analysis of the midge bodies. If radiolabeled material was used, the midges can be oxidized and the concentration of the test material found by scintillation counting (14).

## 12. QUALITY ASSURANCE

- 12.1 Criteria for accepting a test.
  - 12.1.1 At least 80% of control and solvent control midges survive.
  - 12.1.2 Temperature deviation from 22°C does not exceed 3°C.
  - 12.1.3 Dissolved oxygen does not drop below 25% saturation.
  - 12.1.4 pH does not deviate more than one pH unit.
  - 12.1.5 There is at least one toxicant concentration that does not product a statistically significant effect on the biological parameters measured. There is also one concentration which does show significant effect.
  - 12.1.6 Results of analytical measurements on test solutions are not extremely variable. The experimenter has to use good judgment in this regard. Experience indicates that toxicant concentrations may vary by plus or minus 20%, or more for some chemicals especially those which are highly water insoluble. If extreme variation does occur, flow rates may be changed to stabilize concentrations. It is recommended that the test be repeated if the toxicant at the lowest significant effect level is not statistically ( $p < 0.05$ ) different from both the next higher and next lower test concentrations.

## 13. REPORT

- 13.1 The results reported should include the following:
  - 13.1.1 Name of test, investigator, laboratory and date test was conducted.
  - 13.1.2 A brief description of the toxicant, including its source and lot number.

- 13.1.3 The source of dilution water, its chemical characteristics, and a brief description of any pretreatment.
- 13.1.4 A brief description of source of midge, their history, experimental design and summary of methods.
- 13.1.5 Methods used for, and results of, all analysis of test water.
- 13.1.6 Methods used for, and results of, statistical analysis of data.
- 13.1.7 Anything unusual about the test, any deviation from the method and any other relevant information.
- 13.1.8 Raw data.

13.2 Data Retention. All original raw data generated in the study will be provided to the Department of Medicine and Environmental Health staff toxicologist, and the Environmental Sciences Assessment Group GLP file. A copy of the final report (without raw data) will be sent to the Product Acceptability Manager, Environmental Sciences Manager, Environmental Assessment group leader and authors. Data will be retained in MIC GLP file for ten years.

#### 14. METHOD CHANGES

14.1 In the event that modifications of this method are deemed necessary, a written statement of any changes and reasons will be provided by the study director. All agreed upon changes will be expressed in writing, signed and dated by the study director. The signed changes will be appended to the Method and included in the final report.

#### REFERENCES

1. Bender, E. S., 1978. A Glossary of Selected Aquatic Ecological Terms. U.S. Army Special Publication ARCSL-SP-78002.
2. Post, G., 1977. Glossary of Fish Health Terms. American Fisheries Society, Fish Health Section, 48 pp.
3. Standard Operating Procedure. MIC Environmental Sciences. Cleaning Culture Tanks and Test Aquaria. Document number EAS-80-SOP-003.
4. Adams, W.J, W. J. Renaudette, and W. E. Gledhill. 1980. The Toxicity of Santicizer 900D to Early Life Stages of Rainbow Trout (Salmo Gairdneri) ES-80-SS-17.
5. Special Study. MIC Environmental Sciences. Water Quality Characteristics of the Water Samples Used in the N-Building Environmental Laboratory: Part II. Report number ES-78-SS-22.

6. Standard Operating Procedure MIC Environmental Sciences. Procedure for Culturing the Midge Chironomus tentans. Document number EAS-82-SOP-044.
7. Mosher, R. G. and W. J. Adams. 1982. Method for Conducting Acute Toxicity Tests With the Midge Chironomus tentans. MIC Environmental Sciences. Method Report #ES-82-M-7.
8. Standard Operating Procedure MIC Environmental Sciences. Temperature Measurement. Document number EAS-80-SOP-010.
9. Standard Operating Procedure MIC Environmental Sciences. Dissolved Oxygen Determination - YSI-54ARC Operation, Maintenance, and Calibration. Document number EAS-80-SOP-011.
10. Standard Operating Procedure MIC Environmental Sciences. pH Determination. Document number EAS-80-SOP-007.
11. Standard Operating Procedure MIC Environmental Sciences. Alkalinity Measurement. Document number EAS-80-SOP-008.
12. Standard Operating Procedure MIC Environmental Sciences. Hardness Measurement. Document number EAS-80-SOP-009.
13. Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw Hill, New York. 481 pp.
14. Standard Operating Procedure MIC Environmental Sciences. Packard Model B306 Tri-Carb Sample Oxidizer. Calibration, Operation & Maintenance. Document number EAS-80-SOP-021.



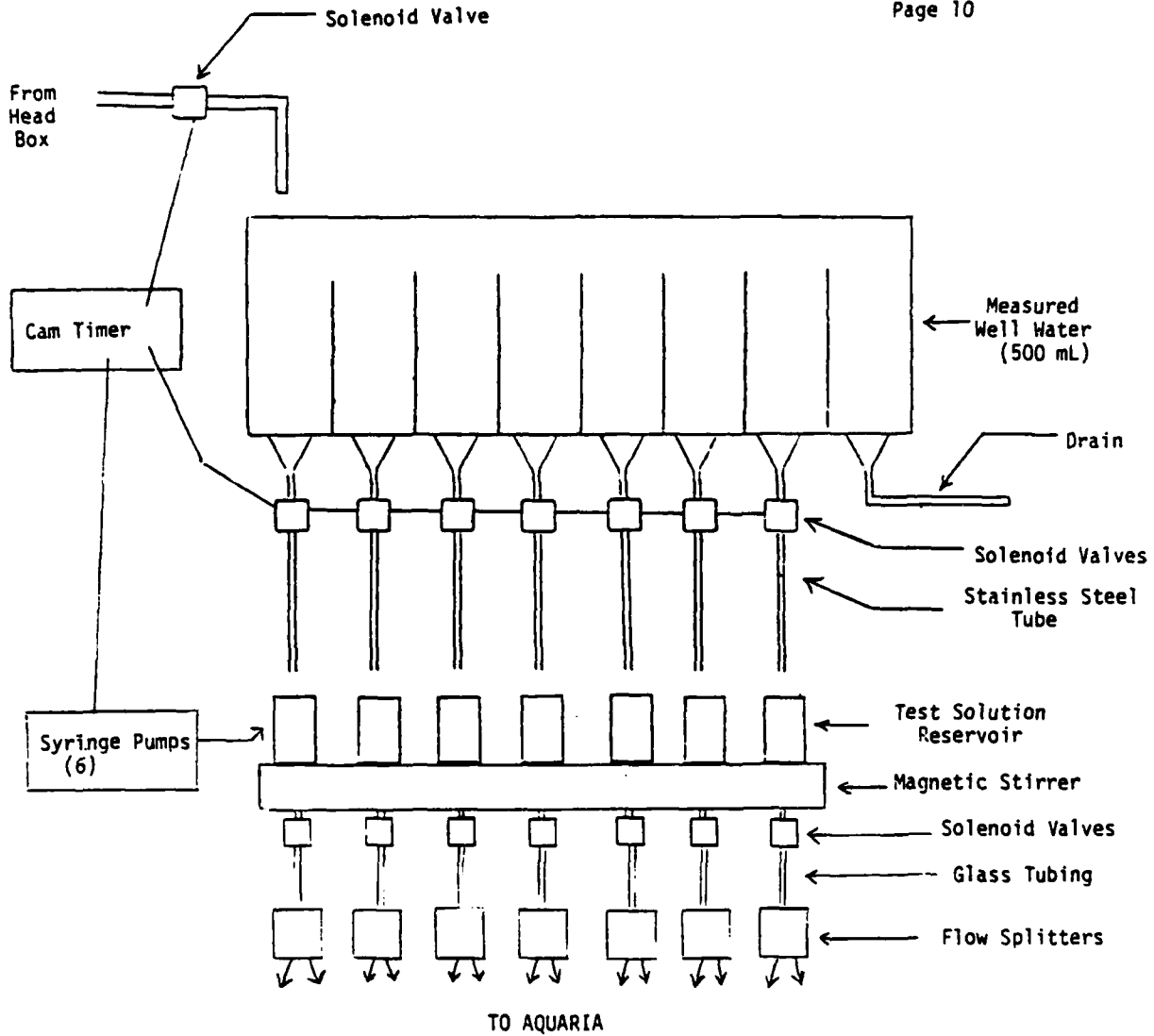


Figure 1. Diagrammatic View of Diluter System Designed to Deliver 250 mL of Test Solution to Each Test Chamber.

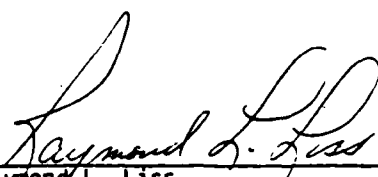
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STANDARD OPERATING PROCEDURE

PROCEDURE FOR CULTURING THE MIDGE CHIRONOMUS TENTANS

Prepared by: Signed Robert M. Mohr Title Research Biologist  
Date June 15, 1982

Approved by: Signed Richard L. Lusk Title Fellow  
Date June 15, 1982

Approved by Group Leader: Signed William J. Adams Title Research Group Leader  
Date 6-21-82

Reviewed for Compliance with ES-GLP Program by: Signed Raymond L. Lewis Title Environ Assmt. Mgr.  
Date 6-21-82

DISTRIBUTION

NO.	DATE	TYPE/DESCRIPTION

REFERENCES

STANDARD OPERATING PROCEDURE FOR CULTURING THE MIDGE, CHIRONOMUS TENTANS

1. PURPOSE AND SCOPE

1.1 Aquatic safety evaluations of chemicals require conducting toxicological studies with aquatic organisms in the laboratory. This procedure provides guidelines on culturing the midge, Chironomus tentans in the laboratory for support of a midge aquatic toxicity testing program.

2. BACKGROUND INFORMATION

- 2.1 It has been recognized that there is a need to include sediment dwelling aquatic organisms when safety testing chemicals that sorb to particles.
- 2.2 Bioaccumulation studies are often a part of chemical safety evaluations and require organisms with a relatively large mass to be conducted conveniently.
- 2.3 The midge, Chironomus tentans (Diptera: Chironomidae), a mosquito-like fly, is recognized as a useful test organism representing the aquatic benthic community in aquatic safety testing.
- 2.4 The immature midge (larva) is worm-like and lives in a case built within soft, flocculent sediments in a variety of aquatic habitats. Larvae can reach a size of 30 mm in length and 25 mg wet weight, making C. tentans one of the larger chironomids.
- 2.5 C. tentans is easily cultured in the laboratory with readily available materials.

3. PROCEDURE FOR CULTURING C. TENTANS

3.1 Quality of Cultures

3.1.1 Care must be taken in culturing to insure healthy organisms are available for testing. This goal can best be met by carefully following the practices that have proven successful.

3.2 Facilities

- 3.2.1 Culture midges in an isolated area or room, free of contamination, and excessive disturbances.
- 3.2.2 Maintain a water temperature of  $22^{\circ} \pm 3^{\circ}\text{C}$  and a wide spectrum light intensity of approximately 100 f.c. in a 16 hour on, 8 hour off regime.

3.3 Water

3.3.1 Culture C. tentans in water of similar quality to the water used in testing.

3.3.2 Natural freshwater dilution water should be uncontaminated, of constant quality, and should meet the EPA specifications for testing aquatic organisms as specified in Methods for Acute Toxicity Tests With Fish, Macroinvertebrates and Amphibians (1975)(3).

3.3.3 St. Peter's well water is used in the Environmental Sciences Laboratories at Monsanto. This water meets EPA and ASTM Standards of quality for natural freshwater dilution water. It has been used successfully to culture and test various aquatic organisms for several years.

### 3.4 Culture Chambers

3.4.1 Rear midges in glass aquaria filled with water to a depth of 8 cm. Size of the aquaria may vary from a minimum of 3 L to a maximum of 19 L depending on the needs of the program. Culture chambers should be covered with Glad Wrap® (polyethylene) to prevent adults from escaping and to exclude other species from entering the cultures.

3.4.2 Culture chambers should be gently aerated with an airstone to maintain a satisfactory dissolved oxygen concentration.

### 3.5 Cleaning

3.5.1 Clean culture vessels before adding midges or eggs following SOP #EAS-80-SOP-003.

3.5.2 Culture water should be changed weekly by the use of a siphon hose to eliminate accumulated waste products. While the water level is down, a razor blade can be used to scrape the sides of the aquarium to remove fungus and algae. Care must be taken to avoid siphoning too much water before cleaning. Fresh water must be added slowly to prevent turbulence.

### 3.6 Midge Source

3.6.1 Obtain eggs or larvae to begin the cultures from another laboratory's culture. Midges can also be obtained from the field.

3.6.2 Establish the correct identity of the midges regardless of the source.

### 3.7 Substrate

3.7.1 C. tentans requires a substrate in which to construct a case. Shredded paper towels have been found to be well suited to this purpose. Strips cut from Scott® or Nibroco brown paper towels are soaked overnight in acetone to remove impurities. The towels are then boiled in three changes of well water until all acetone is removed. A kitchen blender is then used to shred the towels into a pulp. Care is taken to avoid overblending and possible shortening of the wood fibers in the pulp.

- 3.7.2 Rinse the paper towels twice with well water to remove extremely small fibers.
  - 3.7.3 The paper toweling pulp is placed into the water of a culture chamber until a depth of 3 cm is obtained.
  - 3.7.4 Substrate material can be refrigerated until needed. From time to time, new pulp is added to established cultures to replace used up substrate.
- 3.8 Food
- 3.8.1 Chironomus tentans is primarily a filter feeder drawing food particles into its case from the water column. A suspension of "Tetra Conditioning Food Vegetable Diet", obtainable from Beldt's Aquarium, Inc., has been used with good success. One gram of the dry food is mixed into each 10 mL of well water with a kitchen blender. The food should be refrigerated.
  - 3.8.2 For optimal growth of the culture, larvae should be fed twice daily. The amount given depends on the number and size of the larvae. If the water is not clear 3 to 4 hours after feeding, too much was fed. Overfeeding will lead to the growth of fungus in the aquaria and will necessitate more frequent water changes. Therefore, new cultures should receive .5 mL or less of food suspension per feeding water.
- 3.9 Midge Life Cycle
- 3.9.1 C. tentans egg masses hatch 2 or 3 days after deposition in water at temperatures of 19-22°C.
  - 3.9.2 Larval growth occurs in 4 instars of approximately one week each. Under optimal conditions, some larvae will develop into adults 24 to 28 days after egg deposition.
  - 3.9.3 Adults emerge from pupal cases over a period lasting several weeks. Males are easily distinguished from females in that they have large, plumose antennae and a much thinner abdomen with visible genitalia.
  - 3.9.4 Adults are aspirated into a 250 mL Erlenmeyer flask each morning. In late afternoon, approximately 20 mL well water are added to the flask. Eggs are deposited overnight. Every few days, an egg mass should be placed in each culture chamber to perpetuate the culture. Eggs can be stored in a refrigerator to retard development but after 4 or 5 days, viability is greatly reduced.
- 3.10 Culture Logistics
- 3.10.1 Each egg mass contains from 300 to 500 eggs. Two or three fresh egg masses laid gently on the substrate is enough to start a culture chamber.

- 3.10.2 A culture chamber may be productive for several months and can be expected to produce a few adults each day once generations of larvae are staggered.
- 3.10.3 Once a culture becomes unproductive because of worn out substrate or contamination by other organisms, it should be disposed of and a new culture started. Several cultures of different ages should be maintained at any one time as a hedge against unfavorable occurrences.

#### 4. REFERENCES

1. Standard Operating Procedure, MIC Environmental Sciences. Cleaning Culture Tanks and Test Aquaria. Document number EAS-80-SOP-003.
2. Batac-Catalan, Z., and D. W. White. 1981. Creating and Maintaining Cultures of Chironomus tentans (Diptera:Chironomidae). University of Michigan Department of Zoology Report. 4 pp.
3. U. S. EPA 1975. Methods for Acute Toxicity Tests With Fish, Macroinvertebrates and Amphibians. Ecological Research Series, EPA 660/3-75-009, 61 pp.

## CREATING AND MAINTAINING CULTURES OF *CHIRONOMUS TENTANS* (DIPTERA: CHIRONOMIDAE)<sup>1</sup>

Zenaida Batac-Catalan, David S. White<sup>2</sup>

**ABSTRACT:** A modified procedure for continuous culture of *Chironomus tentans* Fabricius, which requires equipment generally available in biological laboratories, is presented. The substrate on which the larvae are reared consists of acetone-treated and boiled paper towels. Liquified vegetable diet is used for more uniform distribution of food in the culture.

Methods exist in the literature for rearing and maintaining cultures of several genera and species of Chironomidae (Biever 1965, Yount 1966; Credland 1973; Downe and Caspary 1973; Gallepp 1979; also see reviews by Fittkau et al. 1976; Merritt et al. 1978) including *Chironomus tentans* (Sadler 1935; Hall et al. 1970). Major difficulties in methodology have been both biological (usually low survivorship) and physical. Even the best methods require construction of special tanks and cages and then may take a considerable period of trial and error through a lack of specific detail in published methods. It is not unusual that a year or more may elapse before some methods produce enough individuals for experimental needs.

*Chironomus tentans*, a hardy species, has proven ideal in ecological and physiological studies, as a toxicological test organism in the laboratory, and may be used as a food source for other aquatic organisms. In designing the methods used, we have relied on basic principles, hints from the literature and three years of our own trial and error. Equipment needed is minimal and generally available in most types of biological laboratories. The methods should be applicable to any of the tube-dwelling, filter feeding or grazing Chironomidae (Leathers 1923).

The quantities given below will create one "continuous" culture in a standard 38 l (10 gal.) aquarium. We do not recommend larger aquariums as they prove to be much less productive per unit area. Aquariums as small as 4 l (1 gal. glass jars) can be used effectively. One culture should yield up to 20 larvae per day. This is equivalent to 180 mg of 3rd instar or 300 mg of 4th larval instar.

**Substrate:** *C. tentans* prefers a soft, flocculent substrate (Sadler 1935) which can be artificially duplicated by ground and shredded paper toweling. To achieve suitable texture and to remove impurities, the paper is soaked in acetone and then boiled. If the chironomid larvae are to be used in tests with

<sup>1</sup>Received October 15, 1981

<sup>2</sup>Great Lakes Research Division and School of Natural Resources, 1081 NU, University of Michigan, Ann Arbor, MI 48109. Contribution No. 327 from the Great Lakes Research Division.



toxic organic compounds, any residual acetone left in the toweling will affect the results even if present only in trace amounts. In this case all the acetone must be removed by keeping the paper in boiling water for at least 48 hours with four or five complete changes of water. It may be desirable to process large batches of paper at one time which then can be kept frozen until needed (R. Mazzone, pers. comm.).

Soak 12 sections (approx. 50 gms) of Scott<sup>®</sup>, Nibroc<sup>®</sup> or an equivalent type of brown paper hand towel (26x10 cm folded two-ply) in enough acetone to keep them wet in a closed glass container for at least 30 minutes. Squeeze out the acetone and replace it with a fresh amount for a second and third 30 minute period. If a Soxhlet acetone extractor is available, the acetone may be reused. Rinse the towels in distilled water or carbon-filtered water four or five times until the strong odor of acetone is removed. Reboil the paper in distilled or carbon filtered water for 1 hour or until most of the color is removed — brown towels will remain a light tan. Finally, cut or tear the towels into smaller pieces and shred to a coarse pulp using a blender.

**Aquarium assembly:** A simple aquarium and adult capture system is given in Fig. 1b. The aquarium is of a standard 38 l (10 gal.) size measuring approximately 26x41x21 cm. The bag (1-2 mm coarse mesh cloth) will effectively contain emerging adults even when loosely fitted to the aquarium. Access to the inside of the bag is through two overlapping flaps that may be closed and fastened by a few pins. Strings attached to the four corners are tied to any fixed structure above the aquarium to hold the bag in place.

**Starting cultures:** In a 38 l aquarium, place 10 l of carbon-filtered or conditioned tap-water (water exposed to the atmosphere and aerated for 3-4 hours.) Add the shredded towel, 1 ml of prepared food (see below), and mix thoroughly. Allow 1 hour of settling time which should produce a substrate layer 2.5-4.0 cm thick. Carefully add enough additional water to create a 3 cm clear layer over the substrate. If any substrate is resuspended during one of the steps, allow time for it to resettle. The air supply to the tank should be at a rate that does not resuspend the substrate. This may be done by suspending an airstone at a level just below the surface of the water. (Fig. 1b). Two or three egg masses obtained by the method below may now be placed very gently on the surface of the substrate.

**Food and feeding:** Several types of food have been used in maintaining larval Chironomidae with varying degrees of success (Biever 1965). We have chosen the following composition because it can be liquified and thus more uniformly distributed in the culture. Food is prepared by blending 20 gm "Tetra<sup>®</sup> Conditioning Food, Vegetable Diet for Tropical Fish" with 200 ml distilled or carbon-filtered water. Prepared food should be kept

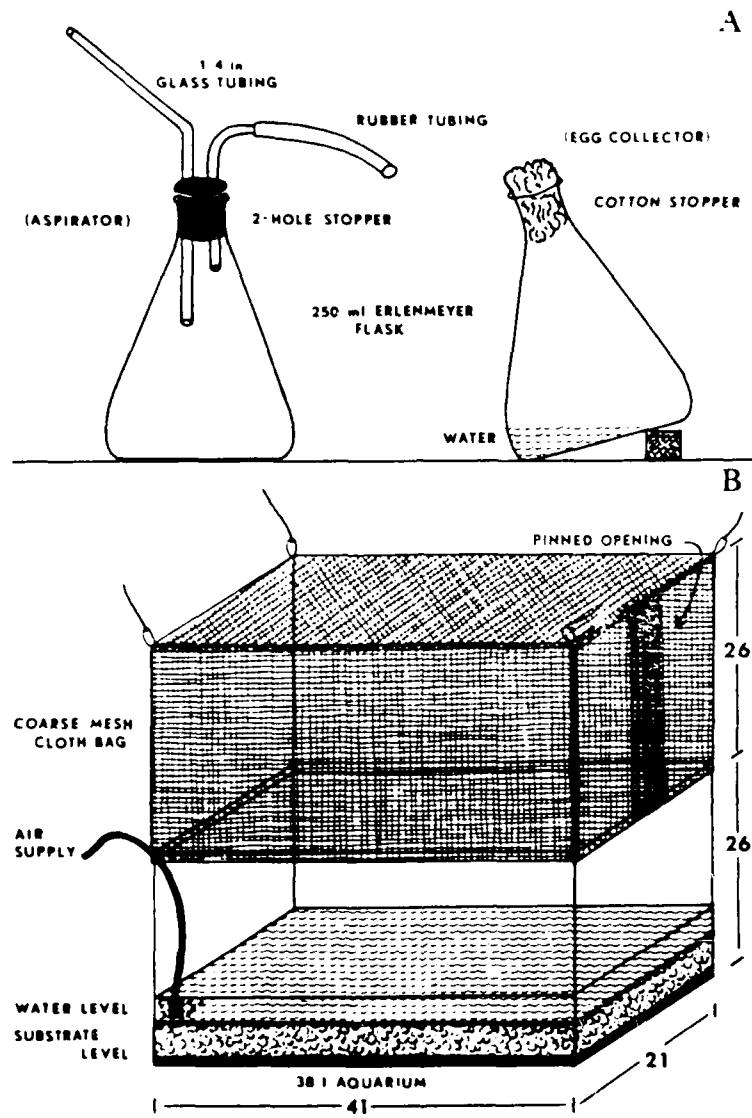


Fig. 1. Equipment employed for forced matings and egg collection of *Chironomus tentans* (A), and oblique view of established culture aquarium with adult capture bag (B), measurements for aquarium and bag are in centimeters.

refrigerated. Shake the mixture well and add about 1 ml at the start of each culture and after every change of water. The amount of food added depends on the density and age of the larvae. If too much food has been added, the water will appear cloudy the next day. If the water remains cloudy, it should be replaced.

**Maintaining cultures:** Because nutrients and byproducts build up quickly, at least part of the water should be changed every 4-7 days. Surface water is siphoned off down to a level just above the substrate. Freshly prepared water plus 1 ml of food is added slowly until the original depth is reached.

**Continuing and starting new cultures:** At 21 C, egg masses hatch 2-3 days after deposition. 1st instars appear in 3-4 days, 2nd instars in 6-8 days, 3rd instars are present after 12-14 days, 4th instars appear around the third week, and adults begin to emerge after 4-5 weeks. The generation of larvae will be continuous to some degree if left undisturbed because a small percentage of the adults will mate and some egg masses will be deposited in the culture. To maintain healthy cultures, a more forced type of mating is recommended. Adults are aspirated into a dry 250 ml Erlenmeyer flask (Fig. 1a) which is then loosely stoppered with cotton (Fig. 1a). Three or four pairs of males and females should produce enough eggs to begin a new culture. Adults are left to mate in the dry flask for several hours, then a volume of 50 ml of conditioned water is gradually added. The flask is set at a slight angle so that most of the water is at one side. Eggs are deposited before dawn, so the age of the mass can be determined. Eggs may be used to restock old cultures, start new ones, or used in experiments that require this life stage. A new egg mass should be added to ongoing cultures every 2-3 days for maximum harvest and emergence rates.

If maintained as above, a culture should be productive for about 6 months. After that time the old culture should be discarded.

#### ACKNOWLEDGMENTS

We wish to thank J. Kawatski of LaCrosse, Wisconsin for supplying the original stock of *Chironomus tentans*. Jarl Hiltunen of the U.S. Fish and Wildlife Service, Ann Arbor, and Clifford Rice and Michael Winnell of the Great Lakes Research Division critically reviewed the manuscript and their comments are greatly appreciated.

#### LITERATURE CITED

- Biever, K.D. 1965. A rearing technique for the colonization of chironomid midges. *Ann. Entomol. Soc. Am.* 64: 1166-1169.
- Credland, P.F. 1973. A new method for establishing a permanent laboratory culture of *Chironomus riparius* Meigen (Diptera: Chironomidae). *Freshwat. Biol.* 3: 45-51.
- Downe, A.E.R., and V.G. Caspary. 1973. The swarming behavior of *Chironomus riparius* (Diptera: Chironomidae) in the laboratory. *Can. Entomol.* 105: 165-171.

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- Fittkau, E.J., F. Reiss, and O. Hoffrichter. 1976. A bibliography of the Chironomidae. Det. KGL. Norske Videnskabers Selskab Museet, Gunneria 26. 177 pp.
- Gallepp, G.W. 1979. Chironomid influence on phosphorus release in sediment-water microcosms. *Ecology* 60: 547-556.
- Hall, D.J., W.E. Cooper, and E.E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. *Limnol. Oceanogr.* 15: 839-928.
- Leathers, A. 1923. Ecological study of aquatic midges and some related insects with special reference to feeding habits. *Bull. U.S. Bur. Fish.* 37: 1-62.
- Merritt, R.W., K.W. Cummins, and V.H. Resh. 1978. Collecting, sampling, and rearing methods for aquatic insects. pp. 13-28. In: R.W. Merritt, and K.W. Cummins. *An introduction to the aquatic insects of North America.* Kendall/Hunt Publ. Co., Dubuque, Iowa. 441 pp.
- Sadler, W.O. 1935. Biology of the midge *Chironomus tentans* Fabricius and methods for its propagation. *Cornell Univ. Agr. Exp. Sta. Mem.* 173: 1-25.
- Yount, J. 1966. A method for rearing large numbers of pond midge larvae, with estimates of productivity and standing crop. *Am. Midl. Nat.* 76: 230-238.
-

Dr. Ray Alden  
Old Dominion University  
Applied Marine Research Laboratory



**Applied Marine Research Laboratory**

PO Box 4692 • Norfolk, VA 23508-8512

March 22, 1985


Thomas Dillon  
Department of the Army  
Waterways Experiment Station  
Corps of Engineers  
Environmental Laboratory  
P.O. Box 631  
Vicksburg, MI 39180

Dear Tom:

Enclosed is a copy of a document in which I have discussed bioassessment techniques which The Applied Marine Research Laboratory has used in dredged material assessment studies. I am afraid that I completed it before I received your letter, so I am not sure whether the format/contents meet all of your needs.

Please let me know if there is any additional information required prior to the meeting.

Sincerely,



Raymond W. Alden III, Ph.D.  
Director  
(804) 440-4195

RWA/reh  
Enclosure

APPLIED MARINE RESEARCH LABORATORY  
OLD DOMINION UNIVERSITY  
NORFOLK, VIRGINIA 23508

**THE ASSESSMENT OF ECOLOGICAL EFFECTS  
OF OPEN WATER DISPOSAL OF DREDGED MATERIALS**

By

Raymond W. Alden III

Prepared for the  
Department of the Army  
Waterways Experiment Station  
Corps of Engineers  
Environmental Laboratory  
P.O. Box 631  
Vicksburg, MI 39180  
Attn: Thomas Dillon

March 1985

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# THE ASSESSMENT OF ECOLOGICAL EFFECTS OF OPEN WATER DISPOSAL OF DREDGED MATERIALS

By

Raymond W. Alden III\*

## INTRODUCTION

The periodic dredging of navigational channels is vital to the maintenance of port systems. Unfortunately, the sediments from urban waterways may be highly contaminated. Pollutants introduced directly or indirectly into the waters of these ecosystems are generally partitioned into, and concentrated in the sediments. Therefore, a problem of major concern to port cities is how potentially toxic dredged materials can be disposed with the least possible ecological damage.

Onshore disposal and landfill management is not feasible in many port systems. In the urbanized setting of most ports, land is at a premium and, therefore, its use for disposal sites is economically unfeasible. Quite often the only open areas in the vicinity of a port are wetlands that should not be filled or impounded due to their ecological value. Therefore, a great deal of attention has been focused upon the possibility of open water disposal of dredged materials.

\*Director, The Applied Marine Research Laboratory, Old Dominion University, Norfolk, VA.

The U.S. Environmental Protection Agency (EPA) and the U.S. Army Corps of Engineers (COE) are responsible for the permitting of ocean disposal operations in the United States. Specific criteria were developed (Federal Register, January 11, 1977) with an Implementation Manual (EPA/COE, 1978) for technical guidelines for evaluating the ecological effects of dredged materials. The guidelines describe a series of lethal bioassay and bioaccumulation experiments which are designed to evaluate the acute toxicity of sediments in order to minimize or prevent severe damage to aquatic ecosystems in the vicinity of the disposal site. Sediments which are shown by these tests to be unacceptable for open water disposal generally are designated for placement in onshore or contained sites where the contaminants will cause the least possible environmental damage.

The purpose of this report is to provide an informal overview of the types of techniques which are available for the evaluation of the acceptability of dredged materials for open water disposal. The conclusions presented in this report are based upon the experiences of The Applied Marine Research Laboratory of Old Dominion University in conducting long-term investigations into dredging/dredged material disposal problems of the Port of Hampton Roads, Virginia. Therefore, the review may not be exhaustive and some of the findings may be unique to the region. However, it is hoped that many of the techniques which have proven useful in our investigations could also be applied to assessment studies in other port cities.

## BIOLOGICAL TESTING

Concerns over the impact of open water dredged material disposal focus upon potential biological effects. Therefore, most of the techniques in use today for dredged material assessment concentrate upon toxicity testing. Many of the previous dredged material quality criteria which were based upon bulk chemical analyses have been abandoned because the concentration of toxins in sediments are not always (in fact, seldom) correlated with toxic effects on the biota exposed to them. The biological availability of toxin/contaminants must be assessed directly by exposing test populations to the dredged materials and evaluating the significance of adverse effects. Several types of biological testing have proven useful: lethal bioassays, sublethal bioassays, bioaccumulation experiments, and multiple-species microcosms. Each of these techniques have certain advantages and disadvantages, but, when employed in a complementary (hierarchical) manner, do provide a very effective and highly defensible assessment of dredged material quality.

### Lethal Bioassays

The Implementation Manual recommends a series of bioassays (toxicity tests) on three dredged material fractions: the liquid phase, the suspended solid phase, and the solid phase. These tests are to be performed on a number of standard test species representing various ecological groups: zooplankton, crustaceans or molluscs, and fish for the liquid and suspended solid phase tests; and crustaceans, infaunal bivalves, and infaunal

polychaetes for solid phase bioassays. However, the number of tests can be reduced, especially if there are a number of dredge sites to be evaluated.

The biological testing protocol developed at the AMRL eliminates all liquid phase bioassays. Experience has shown that liquid phase tests seldom produce significant mortalities in test organisms, even for sediments shown to be toxic in the suspended solid and solid phase experiments. Perhaps of greater importance is the fact that the liquid phase conditions do not have an environmental analog. When dredged materials are disposed in open waters, dissolved chemicals (toxins) are found together with a high suspended solid load, as they are in the suspended solid phase in the laboratory. Therefore, the resources required for liquid phase tests could be better utilized on additional suspended solid phase bioassays, solid phase bioassays, or other biological tests.

Another mechanism for streamlining the toxicity testing procedures is to develop a bioassay screening protocol. Experience has shown that crustaceans are often more sensitive to toxins than fish, molluscs and annelids. Therefore, a screening protocol might include the testing of "worst case" conditions with sensitive test species (e.g. crustaceans) to separate "good" from "bad" sediments. The full strength suspended solid elutriate (as defined by the Implementation Manual) of sediments from a number of sites can be tested at the same time. The toxins in dredged materials are often associated with the organic-rich silt/clay fractions which are found in the suspended solid phase. In

addition, this phase is the fraction to which most organisms are likely to be exposed in the field.

Those sediments which produce little or no mortalities even under highly conservative "worst case" conditions (the majority of the cases tested in most ports) would logically have "passed" the dilution series in the more intensive bioassays described in the Implementation Manual. If deemed necessary, these "non-toxic" sediments (or composites) can be confirmed as acceptable with additional biological tests\* (see below). Those sediments shown to be toxic in the screening tests can be retested in the dilution series or designated for onshore disposal, as determined by economic (cost-benefit) considerations. Experience has shown that "toxic" sediments represent only a small percentage of all materials tested, so a "toxic" designation (unacceptable for open water disposal) based upon the screening tests alone may be economically acceptable. Thus, the screening tests allow resources once devoted to the testing of dilution series to be allocated to more extensive (and cost effective) testing of additional dredge site areas or to complementary biological testing.

#### Solid Phase Bioassays/Bioaccumulation Experiments

Solid phase bioassays are used to confirm the "acceptability" of the screened sediments. These tests provide a longer term exposure of the test species to the dredged materials

\*Should suspended solid phase bioassays be deemed necessary with additional species, a similar screening protocol would be most effective.

for the assessment of toxic effects and allow the evaluation of bioaccumulation effects. Experience has indicated that molluscs are the best indicators of the maximum bioaccumulation potential of toxins in the sediments. However, side by side comparisons between bioassays and conditions simulating those in the field (i.e. microcosms) have indicated that uptake rates observed during these experiments may be somewhat underestimated.

### **Sublethal Bioassays**

If an organism (or a population) is stressed by a toxicant to the point that it can no longer function normally, it may be "ecologically dead," even though it survives the immediate period of exposure (as in the acute conditions of the lethal bioassay). Therefore, there has been concern expressed over chronic sublethal effects of dredged material disposal. Sublethal "condition indices" can be evaluated in the test populations during or after the screening bioassays. We have found that flow-through respiration chambers allow the periodic assessment of the metabolic effects of exposure to the suspended solid phase during the screening experiments with only modest additional costs. Likewise, other sublethal tests can also be made during or after the screening tests: evaluations of osmoregulation capacities (for marine regulators), enzyme activity, energy charge, swimming speed, feeding activity, etc. Sublethal tests have proven useful in characterizing the relative quality of sediments on the peripheries of "toxic" (i.e. lethal) areas. The data from these tests can be used in multivariate statistical models to

objectively classify sediments which have not proven to be acutely lethal in the screening bioassays.

### Microcosm Experiments

Any bioassay or toxicity testing experiment employing standard test species in 10-gallon tanks is subject to the criticism that the conditions are not realistic enough to adequately test the potential lethal effects on endemic biota endemic to a disposal site. Critics of bioassays point out that most standard test species must be relatively hardy in order to be cultured/maintained in the laboratory. Therefore, they may be less sensitive than communities actually living in the vicinity of the disposal site. Moreover, single species static bioassays do not allow an assessment of subtle effects of the dredged materials on such dynamic processes as competition, predation, feeding activity, etc. Therefore, as a final confirmation of the quality of sediments (or sediment composites), multiple species microcosms have been developed. The microcosms have been designed to simulate field conditions as realistically as can be achieved in the laboratory. Natural plankton and benthic communities from the vicinity of the disposal site are introduced into large tanks ( $\sim 1.5\text{m}^3$ ) with controlled circulating and lighting systems to simulate natural currents and photoperiods. The surface to volume ratio of the benthic chamber to the water column is the same as that of the disposal site. A very extensive data set can be accumulated for the comparison of the water quality, plankton community structure, benthic community structure and community respiration/primary production of control and experimental tanks.

The experimental conditions can be designed to simulate conditions in or adjacent to the disposal site (i.e. direct exposure of the benthos to solid or suspended solid fractions). By enhancing the biomass of bioaccumulation indicator species (e.g. molluscs) in certain of the chambers, the biological uptake of toxins can be monitored under more "natural" conditions. In fact, side by side comparisons of clams exposed to dredged materials in bioassays and microcosms have clearly shown that those exposed to the more natural conditions of microcosms display a greater body burden (metals, organic toxins) by the end of the experiments. It is believed that the greater uptake in the microcosms is due to the fact that the currents and/or the natural foods stimulate feeding activities, providing the test species a greater exposure to the toxins (via feeding, or through the gills and integument). On the other hand, the clams in the static bioassay tend to remain closed up (particularly when the sediments are highly contaminated) for longer periods of time.

The data from the microcosms can be effectively analyzed by multivariate statistical models (e.g. MANOVA, discriminant analysis, etc.).

#### **Baseline Monitoring**

Even though the dredged material assessment protocol has been designed to be environmentally conservative, ecosystems in the vicinity of potential dredged material disposal sites should be monitored to provide a baseline data base against which data from trend assessment studies can be compared. If properly



designed, trend assessment monitoring coupled with statistical models based upon an adequate baseline data set can function as an "early warning system" to prevent unforeseen ecological effects from becoming excessively detrimental. A great deal of attention has been devoted by investigators at the AMRL to developing and testing the effectiveness of multivariate statistical models for trend assessment studies. The sensitivity of the monitoring/statistical protocols have been assessed by a series of computer models designed to predict "Minimum Detectable Impacts" (MDI's) for variables (e.g. biological community structure, body burdens, water quality, sediment quality, etc.) examined during the baseline phase of a monitoring program at an open water disposal site. The MDI's are dependent upon natural spatio-temporal variability of baseline data and the intensity of the monitoring effort. The purpose of the evaluation process is to insure that any changes related to dredged material disposal can be detected as being statistically significant before they become ecologically significant.

A second type of "sensitivity analysis" which can be used in assessing the effectiveness of monitoring programs involves data simulation techniques. A computer program has been developed to simulate data sets collected in the field (i.e. containing a variety of non-normal distributions). The program allows the impact of various levels of "impacts" into the simulated data sets, so that the power of various statistical models can be evaluated. Such an approach has allowed the selection of statistical models and ecological monitoring regimes which provide an effective "safety margin" against severe environmental impacts.

Mr. Jim Bajek  
U.S. Army Engineer Division, New England Regulatory Branch  
Waltham, MA

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DEPARTMENT OF THE ARMY  
NEW ENGLAND DIVISION, CORPS OF ENGINEERS  
424 TRAPELO ROAD  
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March 1, 1985

REPLY TO  
ATTENTION OF

Regulatory Branch

Mr. Tom Dillon  
Department of the Army  
Waterways Experiment Station, Corps of Engineers  
Environmental Laboratory  
P.O. Box 631  
Vicksburg, Mississippi 39180

*Tom*  
Dear Mr. Dillon:

This is in response to your January 14, 1985 letter requesting assistance for a possible workshop to be held at St. Paul District. This would involve the development of bioassay and other testing methodologies for assessing the potential impacts of open water disposal of dredged material.

As we recently discussed, I am enclosing a draft copy of a testing guidance manual that is being developed for the New England region. This guidance is currently being refined somewhat to reflect some in-house comments but the basic content should not change substantially. St. Paul District could possibly employ some of the described methodologies even though our manual is intended for marine related disposal actions and St. Paul District is involved only with fresh water disposal.

Our DAMOS mussel watch program should also be considered as a possible disposal site monitoring tool. I will send you a copy of the latest report which should be available in about two or three weeks.

I'm looking forward to participating in this workshop and will await further word from you. I can be reached at FTS 8-839-7213 if there are any questions.

Sincerely,

James Bajek  
Dredged Material Management Section  
Regulatory Branch  
Operations Division

Enclosure

NEW ENGLAND DIVISION ARMY CORPS OF ENGINEERS

REGULATORY BRANCH

DREDGE MATERIAL TESTING GUIDANCE FOR OCEAN DISPOSAL

## INTRODUCTION

The enclosed information explains sediment sampling and testing procedures for permit applicants who propose to dispose of dredged material in open or ocean waters. This guidance manual also includes other administrative requirements for processing applications for Department of the Army approval to dispose of dredged material. The information has been prepared by the New England Division Corps of Engineers in cooperation with Region I of the Environmental Protection Agency (EPA). It will be revised periodically to incorporate modifications to regulatory requirements.

In accordance with Section 227.27 (b) of EPA's Ocean Dumping Regulations and Criteria (Federal Register, Vol. 42, No. 7, Tuesday, 11 January 1977) an Implementation Manual (dated July 1977, Second Printing April 1978) entitled Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters was developed jointly by the Corps of Engineers and EPA to define procedures for evaluating potential environmental impacts associated with ocean disposal of dredged material. The Implementation Manual presents national guidance concerning technical procedures and "is intended to encourage continuity and cooperation between CE Districts and EPA Regions in evaluative programs for Section 103 permit activities." Though the Manual presents detailed procedures for conducting tests required by EPA's Ocean Dumping Criteria, additional guidance is necessary to adapt the procedures to regional

situations. For instance, regional guidance is needed to inform applicants of specific procedural items such as sediment sampling, selection of bioassay organisms and methods of testing.

The guidance presented herein summarizes the tests to be performed and the types of data to be submitted to the New England Division so as to avoid any unnecessary confusion and possible delays in the permit review process through the submission of improper data. This document does not attempt to modify any procedural aspect of the Implementation Manual. Questions regarding any aspect of testing requirements should be directed to:

U.S. Army Corps of Engineers  
New England Division  
Regulatory Branch  
424 Trapelo Rd.  
Waltham, Mass. 02254

REQUIREMENTS

When Applying for Approval

To Dispose of Dredged Material Into Ocean Waters

The first step is to submit a request for authorization to dispose of dredged materials at an open water or ocean disposal site. At that time the following information must be furnished:

1. Up to date information regarding the need for dredging, including quantity of material and area to be dredged, extent of shoaling, interruption or changes in standard operations resulting from shoaling, and any other pertinent information.
2. Current justification and documentation concerning the applicant's investigation of alternative methods and means of disposing the dredged material, as well as the environmental and/or economic reasons for having rejected these alternatives.
3. Two copies of the sediment test data.
4. Two copies of an 8-1/2" x 11" map showing the area to be dredged, the specific location, method and date of sampling.
5. If known, dimensions of the dump vessel (length, width and volume of hopper) and type of dump vessel (split hull or pocket).

If the request is being made under an existing Department of the Army maintenance dredging permit, the permit number should be referenced with a short description of the last dredging performed. Include any past test data for the project area.

6. Identify any known possible sources of contamination to the proposed dredge area. This should include outfalls, spills, surface runoff and any other discharges.

A. Selection of Sediment Sampling Sites

Selecting the proper number and location of sampling sites within the area to be dredged is a crucial step in the testing procedures. As a guideline, a minimum number of 3 sampling sites should be used within the area to be dredged. However, the following factors must be considered when choosing a sampling scheme. These include:

1. The areal extent and heterogeneity of the material to be dredged. If the material varies on the horizontal and/or vertical plane, more sampling sites may be required in order to reflect these differences. If the material varies greatly with depth, or if "new work" dredging is being undertaken, the applicant may be required to obtain core samples and perform separate analyses of differing horizons within samples.



2. Existence of point source discharges in the area to be dredged or other causes for concern such as, historical occurrence of oil spills or other contaminants, outfalls which may affect the area to be dredged including sewage, storm water, agricultural, industrial, municipal, commercial or residential discharges into the waterway. The intent of the ocean dumping criteria is to identify and limit ocean disposal of dredged material which is hazardous to the marine environment. The applicant is required to develop a sampling program which adequately reflects those ends. Notwithstanding these efforts, the sampling scheme is subject to review by the New England Division Corps of Engineers and EPA Region I to insure that these considerations have been fulfilled. The applicant should consult with the Corps Regulatory Branch prior to implementation of any sampling or testing.

B. Types of Sediment Sampling Equipment

← Various types of sediment sampling equipment exist for different situations. The type of equipment employed primarily depends on the composition of the material and the depth of dredging.

1. Tube sampling is usually employed when clayey or silty sediments are encountered and there is a need to determine sample constituents at depth. The samples are obtained by pressing a piston-equipped plastic (butyrate or polycarbonate) tube (approximately 3" in diameter) into the sediment. The employment of plastic tubes are employed to prevent chemical contamination or significant physical alteration of the samples. Tube samples are drained of surface floc, sealed and refrigerated at 2-4°C during shipment to the laboratory. Tube samples should be retained in an upright position, if possible, in order to preserve the in situ integrity of the sediment.

2. A gravity coring device with controlled free fall, consisting of a plastic tube (with or without piston) in a weighted core barrel is used when plastic tubes cannot be directly pressed. These conditions are usually encountered in deep channels where currents or depth make it impossible to press tubes from the surface or divers cannot be employed. Samples obtained by gravity corer are handled in the same manner as all other tube samples before shipment.

3. Drive samples from borings can be used when there is a necessity for obtaining deep samples from hard bottom material provided that a plastic liner is used in the sampling spoon. Samples of the wash should never be analyzed except to check the progress of the boring.

4. Grab sampling is usually employed when:

- a) The material to be dredged is less than three feet thick.
- b) Hard bottom materials are encountered and drive samples are not expedient.
- c) Attempts at tube sampling results in repeated refusal or lack of <sup>s</sup>ample retention.
- d) A surface sample is being purposely sought. If another method is contemplated, coordination with the New England Division should be accomplished prior to field sampling to avoid possible unacceptability of test results.

C. Physical Testing

Physical testing is required for the evaluation of dredged material for ocean disposal and is usually limited to grain size analysis. As a minimum, sediment should be passed through U.S. Standard Sieves #200 and 230 with a distinction made between % fines vs. % granular.

Any core samples taken should be visually inspected for the existence of strata formation. A grain size analysis should be conducted on each distinct layer observed in the material to be dredged.

According to EPA's Ocean Dumping Criteria (Sec.227.13(b)) the material may be excluded from biological testing if one or more of the following conditions prevail:

1. Dredged material is composed predominantly of sand, gravel, rock or any other naturally occurring bottom material with particle sizes larger than silt, and the material is found in areas of high current or wave energy such as streams with large bed loads or coastal areas with shifting bars and channels; or
2. Dredged material is for beach nourishment or restoration and is composed predominantly of sand, gravel or shell with particle sizes compatible with material on the receiving beaches; or
3. The material proposed for dumping is substantially the same as the substrate at the proposed disposal site; and the proposed dredging

site is far removed from existing and historical sources of pollution so as to provide reasonable assurance that such material has not been contaminated by such pollution.

If an applicant wishes to make use of one of the above exclusions, compliance with the criteria must be demonstrated by grain size data and other pertinent, historical, or site specific information as may be required under the circumstances.

#### D. Bulk Chemical Analysis

Bulk analyses are not a specific requirement of the Ocean Dumping testing criteria. It is well documented that chemical constituents found in the sediments show no reliable correlation to uptake in marine animals. As such, the information from this test has limited value. Chemical testing should be performed on samples from within the area to be dredged to determine the presence or absence of constituents of concern. The type and number of samples analyzed depends on many factors such as the extent of dredging (horizontal and vertical), amount of material, possible containment inputs to the area, hydrodynamics of the waterbody, etc. When developing a sampling and testing program, the applicant should keep in mind the primary goal of ~~ob~~<sup>ob</sup>taining samples from enough locations to sufficiently represent the material to be dredged. Prework consultation with NED is encouraged to insure adequacy of results. Table 1 lists constituents normally required to be tested

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However, they are usually conducted to provide additional information such as determining the chemical status of the sediment. This information is very useful for regulatory decisions regarding mitigation or special handling of the material when certain areas indicate differences in contaminant levels.

along with recommended methods and detection limits. Other constituents may be required depending on contaminant source information for the area. It is the applicant's responsibility to inform the Corps of any known contaminants in the project area. Detailed procedures regarding sample handling and testing are described in the EPA/Corps publication, Procedures for Handling and Chemical Analysis of Sediment and Water Samples, printed May 1981 (TR EPA/CE-81-1). Any deviations from this guidance should be authorized by NED Regulatory Branch Personnel before proceeding.

#### E. Elutriate Testing

If the proposed dredge material does not meet the testing exclusions of Section 227.13 (b), liquid phase testing of the material may be required for the same constituents analyzed in the bioaccumulation tests. Appendix C of the manual provides specific guidance in preparing, testing and evaluating the liquid phase (elutriate). Water from the reference site should be used for this analysis. Testing of other constituents depends on the extent of their presence in the project area sediment. Parameters usually include those identified on Table 1. The table also provides recommended testing methods and detection limits.~~table~~.

The elutriate test results are useful for showing what constituents and their respective levels are released from the sediment to the water.

The results are compared to EPA's water quality criteria as well as to the laboratory bioaccumulation data, if needed to further define whether any significant uptake can be attributable to water soluble releases and whether the amounts may be considered undesirable. An "estimation of initial mixing" at the disposal site may also be necessary to determine whether the limiting permissible concentration of the constituent of concern would be exceeded.

#### F. Biological Testing

Dredged material which does not meet the exclusions of Section 227.13(b) discussed previously must undergo bioassay/bioaccumulation testing before it can be considered for ocean disposal. Guidance for performing this testing has been developed jointly by the Corps and EPA and is described in the Implementation Manual which is commonly referred to as the "green book". The lab selected for performing the bioassay/bioaccumulation testing must be completely familiar with the recommended procedures described in the manual.

Bioassay is an abbreviated term for the biological assessment of a test or series of tests that combine a specific amount of the material to be ocean dumped with selected sensitive benthic marine animals to determine the potential for acute toxicity (death) on similar animals at or near the dumpsite perimeter. It is recognized and accepted that benthic animals located directly under the disposal vessel will be

smothered by the discharged material. This is unavoidable and considered an "acceptable" impact on the marine environment. It is the periphery of the disposal area that is of particular concern since this region receives only a slight covering of the dumped material in which many indigenous animals may survive and therefore be subject to potential lethality or bioaccumulation of contaminants from the sediment. It is this condition that the laboratory bioassay procedures are designed to simulate with specific guidance in the Implementation Manual.

Liquid, suspended particulate, and solid phases of discharged material are associated with this type of activity. Currently, NED requires only the solid phase testing with bioaccumulation analysis. There is mutual agreement among the New England Area Federal resource agencies, EPA Region I and NED that the liquid and suspended particulate phase tests do not provide sufficient sensitivity to produce any useful information for permit evaluations and need not be performed for most ocean disposal activities in the New England region. However, NED still retains the option of requiring the liquid and/or suspended particulate phase testing on a case by case basis if it is determined necessary.

Sediment used in the solid phase test must be representative of that to be dredged. If sediment conditions vary substantially within the proposed dredge site, then samples from more than one location may be required for individual testing. Otherwise, samples from several sites (minimum of three) within the proposed dredge area may be composited for one test.

Sediment from reference and control areas must be obtained for comparative testing. Dumpsite water or artificial seawater (28 to 30 pp<sup>t</sup><sub>A</sub> salinity) is acceptable for all test aquaria. Bioassay test water must be maintained at 20°C±2° unless directed otherwise by New England Division. The tanks should use a filtered flow through design which replaces the volume of each aquaria at least once every four hours. The static design with 75% replacement of the seawater volume every 48 hours is satisfactory if a continuous-flow system is not available.

#### REFERENCE SEDIMENT

Reference sediment must be obtained from the natural environment near and similar to dumpsite material but not influenced by previous disposal activities. The purpose of the reference sediment in the lab test is to simulate conditions at the dumpsite if disposal of dredged material had not occurred. This will allow comparison with ~~with~~ dredged material for predicting possible degradation within the proposed disposal area. The reference sediment results are compared to that of the proposed dredge material and if necessary a statistical analysis is performed to determine whether the dredge material data is significantly different than the reference material results. The reference material used for a particular bioassay must be subjected to bulk sediment analysis. Specific parameters are listed on Table 1.



Location: Reference sediment will be collected at specific locations outside the dumpsites as shown on the attached charts. The exact locations may change from time to time depending on existing conditions. The Corps office should be consulted prior to initiation of reference material sampling.

#### CONTROL SEDIMENT

Control sediment for the solid phase bioassay is used to determine the health of the organisms relative to the testing conditions during the testing period. When the control mortality exceeds 10%, all solid phase bioassay testing must be repeated because the results would be suspect of being influenced by something other than the sediment. Only three control replicate aquaria need be used since statistical analyses is not performed on those results.

*three replicate tanks?  
sample into a lot.*

Location: Control sediment can be collected from any uncontaminated intertidal estuarine area and may consist of fine grained or coarse (sand) material. The sediment should be periodically checked for chemical constituent levels to insure its uncontaminated nature. This data must be furnished to NED.

Organisms surviving the solid phase bioassay tests in the control sediment must be placed in sediment-free water for a minimum of two days to purge digestive tracts of sediment. The organisms must then be

immediately frozen and retained for possible post-test bioaccumulation analysis. They should be maintained for a minimum of six months after the results have been reviewed by the Corps and EPA and the project public notice issued.

The following species are considered to be appropriate sensitive marine organisms for solid phase bioassay testing in the New England region:

Nereis sp.<sup>1</sup> or Neanthes sp.<sup>1</sup> - Infaunal Polychaete  
(Sandworm)

Mercenaria mercenaria - Infaunal Bivalve  
(Hard clam)

Palaemonetes sp.<sup>1</sup> - Crustacean  
(Grass shrimp)

The solid phase test is performed for a ten day period. At the end, if there is greater than 90% survival of combined species in the control aquaria, <sup>then?</sup> the number of test aquaria survivors can be statistically compared to the surviving numbers in the reference aquaria. This will show any potential for acute toxicity from the dredge material in question. Refer to Appendix F of the Implementation Manual for further details on statistical analysis.

<sup>1</sup>Actual genus and species used must be properly identified.

The bioaccumulation analysis is performed by measuring and statistically comparing body burden levels of certain contaminants on the test and reference aquaria survivors. Cadmium, mercury, petroleum hydrocarbons (PHC's), PCB's and DDT are the main constituents of concern. However, other contaminants may be analyzed if believed to be available in substantially high concentrations. For PHC analysis, the levels of aromatic hydrocarbons must be measured and recorded separately from the aliphatics. For regulatory purposes, the aromatic level is of more concern and a statistical comparison of any uptake between test and reference organisms is required. Representative organisms from the stock populations must be tested for the same constituents as the test and reference organisms. There should be a minimum of three replicate analyses for each control species per constituent and five replicate analyses for each test and reference species per constituent

The required detection limits for each constituent are listed below:

<u>Constituent</u>	<u>Required Detection Limit</u>
Hg	0.20 mg/kg
Cd	0.25 mg/kg
PCB's	0.04 mg/kg
DDT	0.02 mg/kg
PHC's	0.10 mg/kg

G. Data Analyses

Generally, the guidance presented in the Implementation Manual should be followed with the exception that the ANOVA analysis be performed instead of the Students t-test when comparing two sets of samples. However, when the ANOVA method is used it must be insured that the data has been found to be homogeneous using Cochran's determination. Otherwise, a nonparametric analysis is necessary.

H. Laboratory Quality Assurance

It is extremely important that the results obtained from the laboratory are reliable and represent the sediment/organism response as accurately as possible. This can be done only through a strict quality control program during sediment sampling, handling, transportation, storage and laboratory testing. Consequently, the applicant must insure that the selected lab is fully capable of performing these services in a competent manner.

NED, and EPA Region I, require that the following be included in all bioassay testing programs:

1. Chain of custody procedures for sampling, handling and storing samples.
2. Use of all procedures described in Procedures For Handling and Chemical Analysis of Sediment and Water Samples<sup>(1)</sup>

Routine use and documentation of intra-laboratory quality control practices as recommended in the EPA manual Handbook for Analytical Quality Control in Water and Wastewater Laboratories<sup>(2)</sup>. These practices must include use and documentation of internal quality control samples.

The laboratory facilities, data and records are subject to periodic inspection by the New England Division and EPA Region I personnel.

(1) This technical report (EPA/CE-81-1) may be obtained by contacting: Environmental Laboratory  
U.S. Army Engineer Waterways Experiment Station  
P.O. Box 631  
Vicksburg, Miss. 39180

(2) This publication (EPA-600/4-79-019) may be obtained by contacting: Environmental Monitoring and Support Laboratory  
U.S. EPA Office of Research and Development  
Cincinnati, Ohio. 45268

New England Division personnel are available to answer questions which may arise in the course of the testing process. Questions may be addressed to:

U.S. Army Corps of Engineers  
New England Division  
Regulatory Branch  
424 Trapelo Rd.  
Waltham, Mass. 02254  
Phone: (617) 647-8213 or  
Toll-free lines: 1-800-343-4789 Outside Massachusetts  
Toll-free lines: 1-800-362-4367 Inside Massachusetts

Additional copies of this Guidance Manual may be obtained from the  
aforementioned address.

Copies of the EPA/COE Implementation Manual Criterion Ecological  
Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters;  
Implementation Manual for Section 103 of the Public Law 92-532 (Marine  
Protection, Research and Sanctuaries Act of 1972), July 1977 (Second  
Printing April 1978) may be obtained by writing to:

Environmental Effects Laboratory  
U.S. Army Engineer Waterways Experiment Station  
P.O. Box 631  
Vicksburg, Mississippi 39180

ATTN: Publications Office  
or calling-(601) 636-3111-Publications Office

Table 1

Parameter	BULK SEDIMENT TEST		ELUTRIATE TEST	
	Suggested Method	Detection Limit	Suggested Method	Detection Limit
Volatile Solids	NED	1%	-	-
Water	-	1%	-	-
Total Kjeldahl Nitrogen (TKN)	Block Digested, Automated	50 ppm	-	-
Oil and Grease	Hexane extract Gravimetric	0.5%	Solvent Extract Gravimetric	5 ppm
Mercury - Hg	AD, Flameless AAS	0.1 ppm	AD, Flameless AAS	0.5 ppb
Lead - Pb	AD, AAS	20 ppm	SE, AAS	10 ppb
Zinc - Zn	AD, AAS	20 ppm	SE, AAS	10 ppb
Arsenic - As	Gaseous Hydride AAS	1 ppm	Gaseous Hydride AAS	10 ppb
Cadmium - Cd	AD, AAS	2 ppm	SE, AAS	10 ppb
Chromium - Cr	AD, AAS	20 ppm	SE, AAS	10 ppb
Copper - Cu	AD, AAS	20 ppm	SE, AAS	10 ppb
Nickel - Ni	AD, AAS	30 ppm	SE, AAS	10 ppb
PCB's	Extraction, GC	1 ppb	Extraction, GC	0.001 ppb .02

NED - New England Division Method. Sample heated to 350-400°C

AD - Acid Digestion

SE - Solvent Extraction

AAS - Atomic Absorption Spectrophotometry GC - Gas Chromatography

Reference: Plumb, R.H., Jr. 1981. "Procedure for Handling and Chemical Analysis of Sediment and Water Samples," Technical Report EPA/CE-81-1, prepared by Great Lakes Laboratory, State University College at Buffalo, Buffalo, N.Y., for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material. Published by the U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

Bulk sediment metals and PCB data should be expressed in ppm or ppb based on dry weight of sample. Elutriate data should be expressed as ppb (weight per unit volume). Additional parameters may be requested if there is concern for special contaminants in the area.

## DREDGE MATERIAL EVALUATION GUIDANCE

1. Purpose: This guidance is provided to aid in the initial evaluation of proposed discharges of dredge or fill material, and to determine the need for testing.
2. Applicability: This guidance applies to discharges of dredged material subject to Section 404 of the Clean Water Act, and Section 103 of the Ocean Dumping Act. All activities within the "baseline"<sup>1</sup> must be evaluated under the guidance of the Clean Water Act (404(b) Guidelines), and those activities seaward of the "baseline" must be evaluated under the Ocean Dumping Act (103 Criteria). In Long Island Sound open water discharges in excess of 25,000 cubic yards must be evaluated under the Ocean Dumping Criteria.
3. Preliminary Screening: In order to determine the scope and need of testing to evaluate a proposed open water discharge the following information must be considered:
  - a. Potential routes of contaminant introduction. (Refer to maps, aerial photographs; make field inspections where necessary)
    - (1) Natural drainage.
    - (2) Outfalls.
    - (3) Hydrology of water body.
  - b. Test data from previous sampling in proximity to the project, includes data from:
    - (1) Federal projects.
    - (2) State and local projects.
    - (3) Private projects.
    - (4) Other studies in the area.
  - c. Historic introduction of contaminants.
    - (1) Knowledge of past types of activities in and around the waterbody and their bearing on possible contaminant introduction.
    - (2) EPA listings.
  - d. Documented Spills of Substances.

<sup>1</sup>Territorial Sea Baseline or Baseline - The delineation of the shoreward extent of the territorial sea. Mean low water is the usual limit. Where islands, bays, and estuaries are present the baseline may exclude such areas from the territorial seas. NOS charts depict the baseline. Figure I shows the general location of the baseline in New England waters.

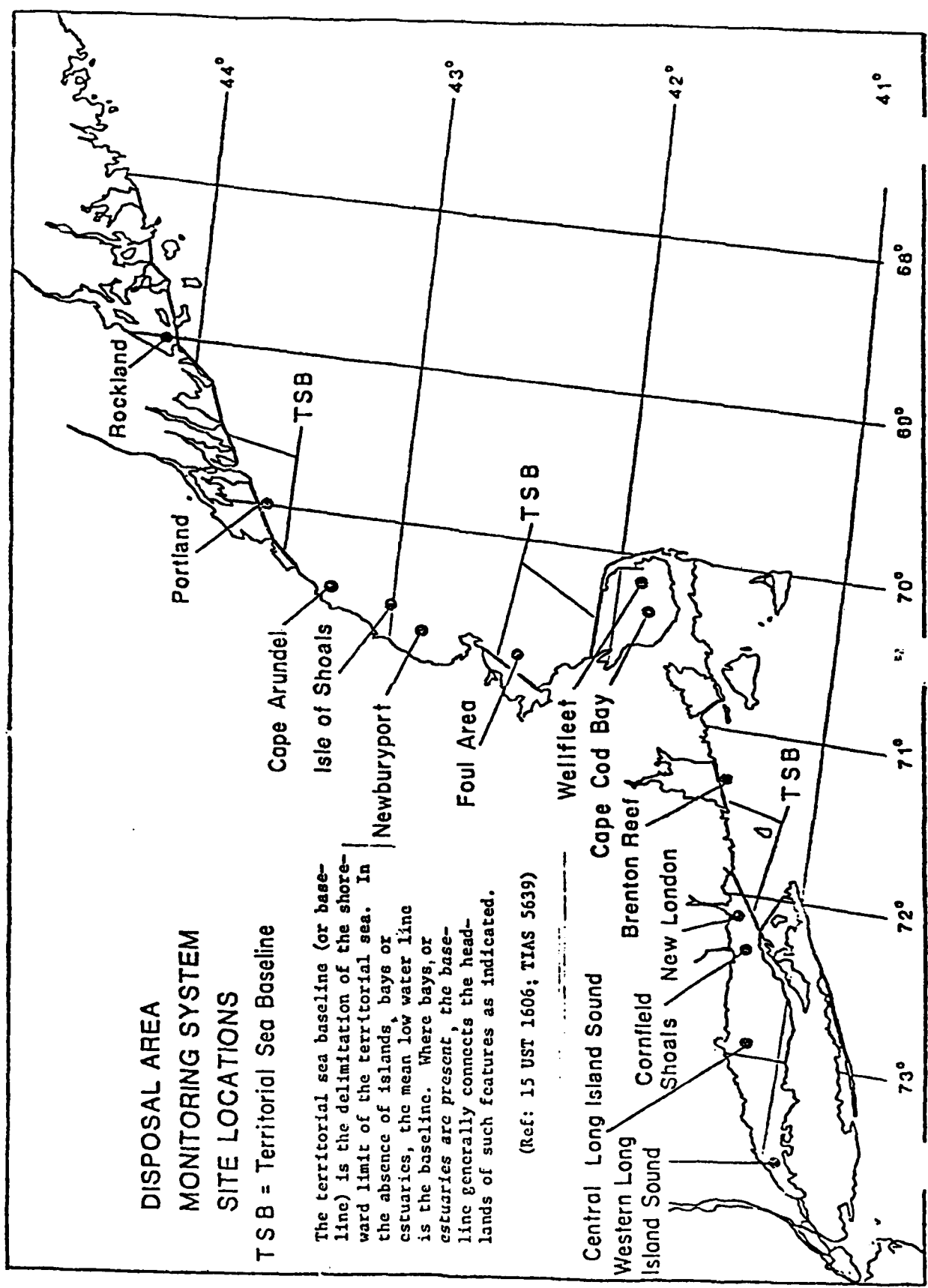


**DISPOSAL AREA  
MONITORING SYSTEM  
SITE LOCATIONS**

**T S B = Territorial Sea Baseline**

The territorial sea baseline (or base-line) is the delimitation of the shoreward limit of the territorial sea. In the absence of islands, bays or estuaries, the mean low water line is the baseline. Where bays, or estuaries are present, the baseline generally connects the headlands of such features as indicated.

(Ref: 15 UST 1606; TIAS 5639)



DREDGE MATERIAL EVALUATION GUIDANCE

- (1) Pollution Incident Reporting System (Oil).

U.S. Coast Guard  
1st District, Boston (223-6915)  
3rd District, Gov. Is., NY (8-664-7152)

NOTE: Lat/Long coordinates are needed when requesting information.

- (2) EPA Listings.

- e. Recent municipal, agricultural and industrial waste loading.

- (1) NPDES Permit Records.

Contacts: CT - DEP Water Compliance Unit (8-641-2211 x7167)  
RI - DEM (401-227-2234)  
MA - DEQE - Division of Land & Water Use (727-4794)  
NH - Water Supply & Poll. Control Com. ((603) 272-3503)  
ME - DEP ((207) 868-2111)  
EPA - Permits Section (223-5061)

- (2) EPA Listings.

- f. Source and previous use of fill materials.

- (1) Corps Permits.

- (2) Other Above References.

- g. Natural mineral deposits.

- (1) U.S. Bureau of Mines. (8-634-7131)

- (2) U.S. Geological Survey. (8-860-6446)

4. Adequacy of Existing Information. Consider and weigh available information in terms of proximity, relevancy and quality of data seeing that:

a. The available data are from points reasonably close to and not isolated from the project by any impermeable physical barriers.

b. There is no reason to suspect high, short-distance variability.

c. The hydraulic regime at the project site is similar to that from which the existing data was obtained.

5. Additional Testing:

## DREDGE MATERIAL EVALUATION GUIDANCE

If a judgement as to acceptability cannot be made with existing information, additional testing may be required (see flow diagram). At present, the initial screening test for open water discharges is the bulk sediment analysis. Bioassay/bioaccumulation and/or elutriate testing may also be required. The appropriate test to assess upland discharges with runoff into the "waters of the United States"<sup>2</sup> is the elutriate test. Various extraction tests including the Extraction Procedure Toxicity (EP Tox.) test can be used for determining potential contamination of ground water caused by subterrestrial leaching from upland dredge material disposal sites.

### 6. Sampling:

a. The goal in choosing the sampling pattern and technique is to obtain a representative sample of the proposed discharge material. The areal extent of the project, quantity of material and sources of contamination are prime considerations in determining the sampling plan. The sampling technique is determined by considering the penetration depth required and general sediment characteristics. The availability of equipment, sampling costs and the type of testing may limit the available options.

b. Tube sampling is usually employed when clayey or silty sediments are encountered and there is a need to determine sample constituents at depth. The samples are obtained by pressing a piston-equipped plastic (butyrate or polycarbonate) tube (2-7/8" I.D.-3" O.D.) into the sediment. Plastic tubes are employed to prevent chemical contamination or significant physical alteration of the samples. Tube samples are drained of surface floc, sealed, and refrigerated at 2-4°C during shipment to the laboratory.

c. A gravity coring device with controlled free fall, consisting of a plastic tube (with or without piston) in a weighted core barrel is used when plastic tubes cannot be directly pressed. These conditions are usually encountered in deep channels where currents or depth make it impossible to press tubes from the surface or divers cannot be employed. Samples obtained by gravity corer are handled in the same manner as all other tube samples before shipment.

d. When there is a necessity for obtaining deep samples from hard bottom material drive samples from borings can be used provided that a plastic liner is used in the sampling spoon. Samples of the wash should never be analyzed except to check the progress of the boring.

e. Grab sampling is usually employed when:

- (1) The material to be dredged is less than three feet thick.

<sup>2</sup>Such discharges are covered under a Nationwide permit provided the State has issued a Water Quality Certification (33 CFR 330.5(a)(16)).

## DREDGE MATERIAL EVALUATION GUIDANCE

(2) Hard bottom materials are encountered and drive samples are not expedient.

(3) Attempts at tube sampling results in repeated refusal or lack of sample retention.

(4) A surface sample is being purposely sought.

f. Where open water or ocean dumping is being considered, it is most desirable to use dumpsite water in order to assess the water quality impacts at the disposal area. If water samples from the disposal area are not available, dredge site water samples may be used. Disposal site water should be taken in equal amounts from about one foot below the surface at middle depth and about three feet above the bottom; subsamples from each of these depths are composited into one sample for testing. Samples are stored in one-gallon plastic containers and refrigerated at 2-4°C during shipment. Dredge site water samples for elutriate testing are taken at about one foot below the surface at each corresponding sediment sample site. Samples destined for PCB testing should be stored in one-gallon glass containers.

### 7. Testing

#### Physical and Chemical Testing.

a. In addition to grain size analysis (include U.S. Standard Sieves #200 and 230), the standard parameters to be tested for include the following:

DREDGE MATERIAL EVALUATION GUIDANCE

Parameter	BULK SEDIMENT TEST		ELUTRIATE TEST	
	Suggested Method	Detection Limit	Suggested Method	Detection Limit
Volatile Solids	NED	1%	-	-
Water	-	1%	-	-
Total Kjeldahl Nitrogen (TKN)	Block Digested, Automated	50 ppm	-	-
Oil and Grease	Hexane extract Gravimetric	0.5%	Solvent Extract Gravimetric	5 ppm
Mercury - Hg	AD, Flameless AAS	0.1 ppm	AD, Flameless AAS	0.5 ppb
Lead - Pb	AD, AAS	20 ppm	SE, AAS	10 ppb
Zinc - Zn	AD, AAS	20 ppm	SE, AAS	10 ppb
Arsenic - As	Gaseous Hydride AAS	1 ppm	Gaseous Hydride AAS	10 ppb
Cadmium - Cd	AD, AAS	2 ppm	SE, AAS	10 ppb
Chromium - Cr	AD, AAS	20 ppm	SE, AAS	10 ppb
Copper - Cu	AD, AAS	20 ppm	SE, AAS	10 ppb
Nickel - Ni	AD, AAS	30 ppm	SE, AAS	10 ppb
PCB's	Extraction, GC	1 ppb	Extraction, GC	0.001 ppb

NED - New England Division Method. Sample heated to 350-400°C  
 AD - Acid Digestion SE - Solvent Extraction  
 AAS - Atomic Absorption Spectrophotometry GC - Gas Chromatography

*changed to 0.02 ppb*

Reference: Plumb, R.H., Jr. 1981. "Procedure for Handling and Chemical Analysis of Sediment and Water Samples," Technical Report EPA/CE-81-1, prepared by Great Lakes Laboratory, State University College at Buffalo, Buffalo, N.Y., for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material. Published by the U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

Bulk sediment metals and PCB data should be expressed in ppm or ppb based on dry weight of sample. Elutriate data should be expressed as ppb (weight per unit volume). Additional parameters may be requested if there is concern for special contaminants in the area.

b. Biological Testing. The laboratory bioassay tests attempt to simulate the disposal of dredged material in the marine environment and assess the potential for acute toxicity and chronic effects on the benthic community at or near the dumpsite boundary. Liquid, suspended particulate and solid phases of discharged material are associated with this type of activity. Currently, NED requires only the solid phase testing with bioaccumulation analysis. There is mutual agreement among the Federal resource agencies, EPA Region I and NED that the liquid and suspended particulate phase tests do not provide sufficient sensitivity to produce any useful information for permit evaluations and need not be performed.

## DREDGE MATERIAL EVALUATION GUIDANCE

Sediment used in the solid phase test must be representative of that to be dredged. If sediment conditions vary substantially within the proposed dredge site, then samples from more than one location may be required for individual testing. Otherwise, samples from several sites (minimum of three) within the proposed dredged area may be composited for one test.

Sediment from a reference area near the proposed dumpsite as well as from a control area must also be obtained for comparative testing. Dumpsite water or artificial seawater (28 to 30 ppm salinity) is acceptable for all test aquaria. Bioassay test water must be maintained at  $20^{\circ}\text{C} \pm 2^{\circ}$  unless directed otherwise by New England Division.

The following species are considered to be appropriate sensitive marine organisms for solid phase bioassay testing in the New England region:

Nereis sp.<sup>3</sup> or Neanthes sp.<sup>3</sup> - Infaunal Polychaete  
(Sandworm)

Mercenaria mercenaria - Infaunal Bivalve  
(Hard clam)

Palaemonetes sp.<sup>3</sup> or Crangon sp.<sup>3</sup> - Crustacean  
(Grass shrimp) (Sand shrimp)

The solid phase test is performed for a ten day period. At the end, if there is greater than 90% survival of combined species in the control aquaria, the number of test aquaria survivors can be statistically compared to the surviving numbers in the reference aquaria. This will show any potential for acute toxicity from the dredge material in question. Refer to Appendix F of the Implementation Manual for further details on statistical analysis.

The bioaccumulation or chronic toxicity analysis is performed by measuring and statistically comparing body burden levels of certain contaminants on the test and reference aquaria survivors. Cadmium, mercury, petroleum hydrocarbons, PCB's and DDT are the main constituents of concern. However, other contaminants may be analyzed if believed to be available in substantially high concentrations. Table G1 (Implementation Manual) references standard analytical methods for tissue analysis for these constituents. All bioaccumulation data should be expressed in ppm, wet weight and analyzed in accordance with Appendix G of the Implementation Manual.

c. Relative Costs of Testing: These are approximate price guidelines as of yearend 1982. It should be emphasized that these costs are highly

<sup>3</sup>Actual genus and species used must be properly identified.

DREDGE MATERIAL EVALUATION GUIDANCE

variable among laboratories and that cost comparisons should be sought before the final selection.

- |   |                |
|---|----------------|
| (1) Bulk Sediment, per set of analyses as described above;<br>(sampling not included) | \$250-450      |
| PCB's (additional per sample)   | \$200-300      |
| (2) Elutriate Tests (per each set)  | \$1,000-1,500  |
| PCB's (additional per sample)   | \$600-1000     |
| (3) Solid Phase Bioassay with Bioaccumulation,<br>sampling included <sup>4</sup>      | \$6,000-10,000 |

8. Enveloping:

The preliminary screening process discussed in paragraph 3 above may be expanded to assess a larger area which may encompass a Federal project as well as several private projects. In this context the process is referred to as "enveloping" and any tests which may be required would be designed to assess the entire envelope.

Regulation 33 CFR 322.5(c)(1) and 325.2(b)(4) require that the Corps consider private dredging interests during the planning and assessment of Federal navigation projects. This should involve early coordination with Federal, State and local authorities as well as the general public by means of public notice. With this notification process we should describe the nature, estimated amount and frequency of known and anticipated related dredging to be conducted by various non-Federal interests in the general area.

To conform with the intent of these requirements NED has developed a practice which involves early interagency participation. The initial step is to gather all existing data and determine the extent to which anticipated projects in the area could be included for an overall assessment. An envelope is designed to include all areas of similar sediments which are in proximity to the Federal Project. Testing is performed in both Federal and non-Federal areas. Next, the area is described by public notice to gather further information and comments to determine if the boundary limits should be revised. No future individual public notices will be issued unless additional information and/or comments are determined necessary after the first notice. The intent is to use a single public notice and environmental assessment for the Federal Project which also includes potential non-Federal users within the envelope described.

<sup>4</sup>Since these tests are very time consuming and costly the selected testing lab should consult with NED before sampling and testing to ensure they are performed correctly.

## DREDGE MATERIAL EVALUATION GUIDANCE

The envelope limits are flexible and additional testing within the area can be required at any time if new information requires it. Continuous coordination by NED's Regulatory Branch will be maintained with state, EPA and other resource agencies after an envelope has been formulated. These agencies will be notified at Regulatory Branch Joint Processing meetings whenever an application is received to dredge within the area. This will allow ample time for additional site specific research and coordination with these agencies. The envelope limits will be changed whenever warranted. This could occur because of changing conditions or a newly identified important resource within the subject area.

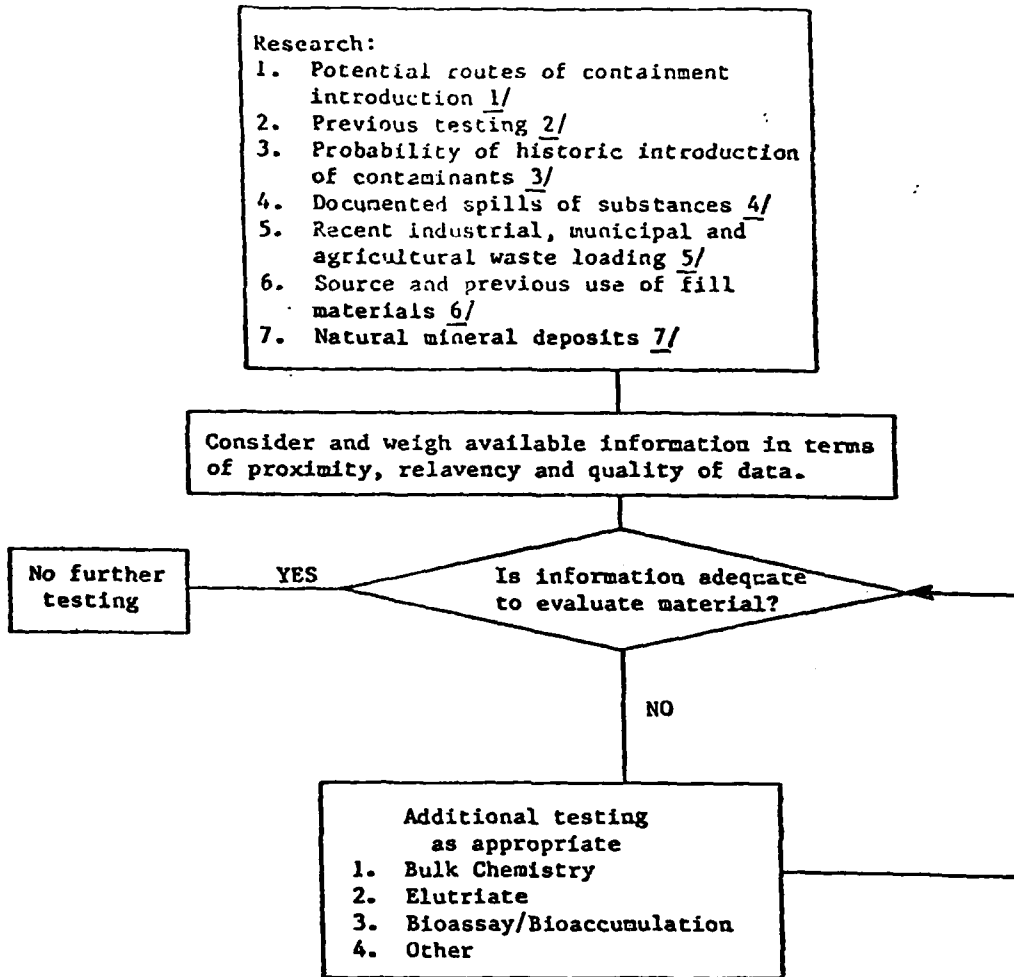
The envelope concept does not automatically eliminate the requirement for further testing within the area. The Corps will always reserve the authority to require individual testing whenever available information shows it is necessary.

The following flow chart outlines the major considerations NED follows in researching and evaluating an area to determine if there is sufficient information such that no further testing would be necessary. This methodology can be used for an individual site or enveloped area.

9. Additional guidance or revisions to the above will be circulated as appropriate.

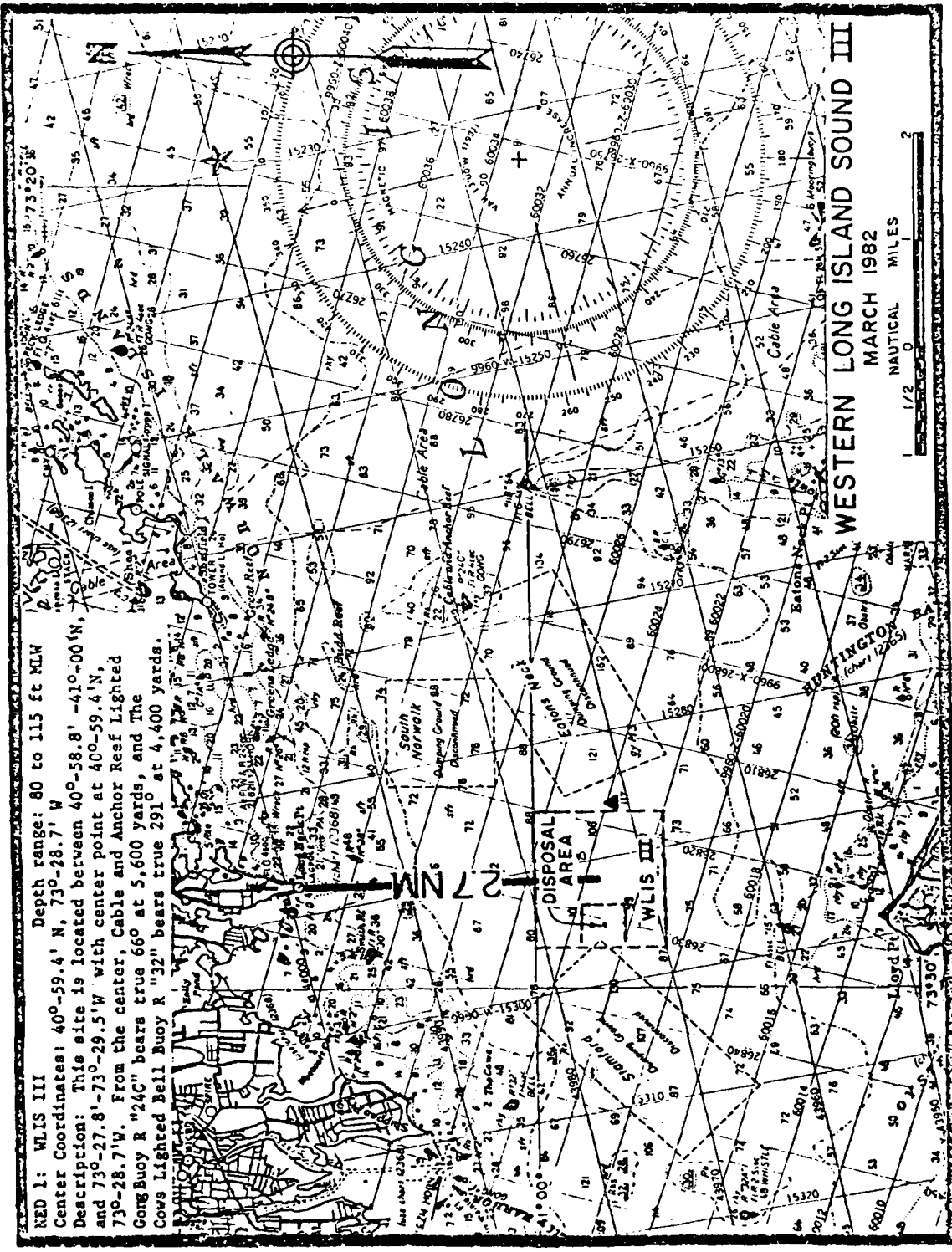


DREDGED MATERIAL EVALUATION GUIDANCE



Footnotes:

- 1/ Maps, aerial photos, field inspections.
- 2/ Testing for Federal, State, private projects.
- 3/ Knowledge of past industrial and shipping activity in waterbody or in surrounding watershed. EPA listings.
- 4/ EPA listings.
- 5/ a. NPDES Permit Records.  
b. EPA listings.
- 6/ Corps permits, other above references.
- 7/ USBM, USGS.

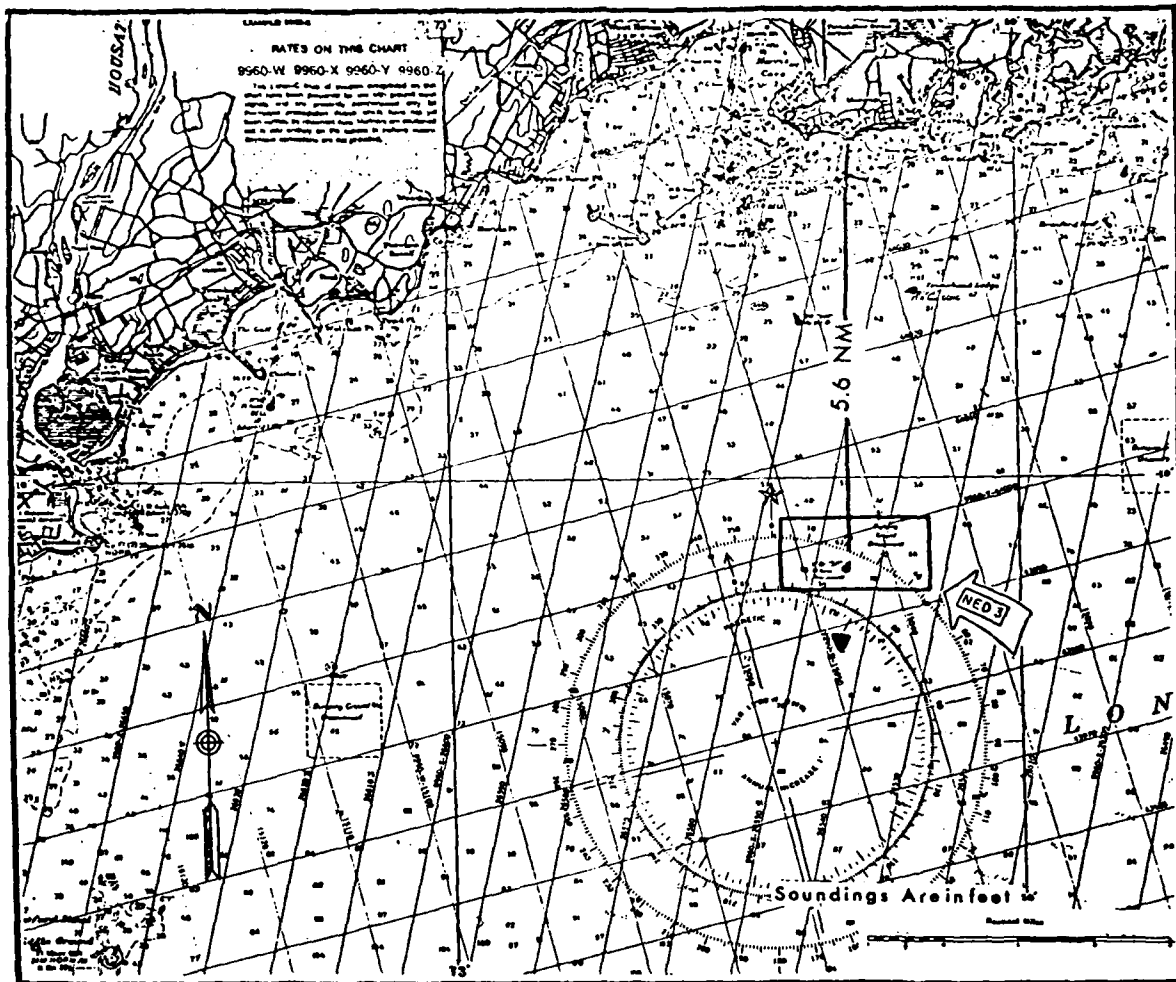


**RED 1: WLIS III** Depth range: 80 to 115 ft MLW  
 Center Coordinates: 40°-59.4' N, 73°-28.7' W  
 Description: This site is located between 40°-58.8' -41°-00' N, and 73°-27.8' -73°-29.5' W with center point at 40°-59.4' N, 73°-28.7' W. From the center, Cable and Anchor Reef Lighted Gong Buoy R "24C" bears true 66° at 5,600 yards, and The Cows Lighted Bell Buoy R "32" bears true 291° at 4,400 yards.

**WESTERN LONG ISLAND SOUND III**  
 MARCH 1982  
 1/2 NAUTICAL MILES

Ref. N.O.S. CHART 12363, Jan. 1981

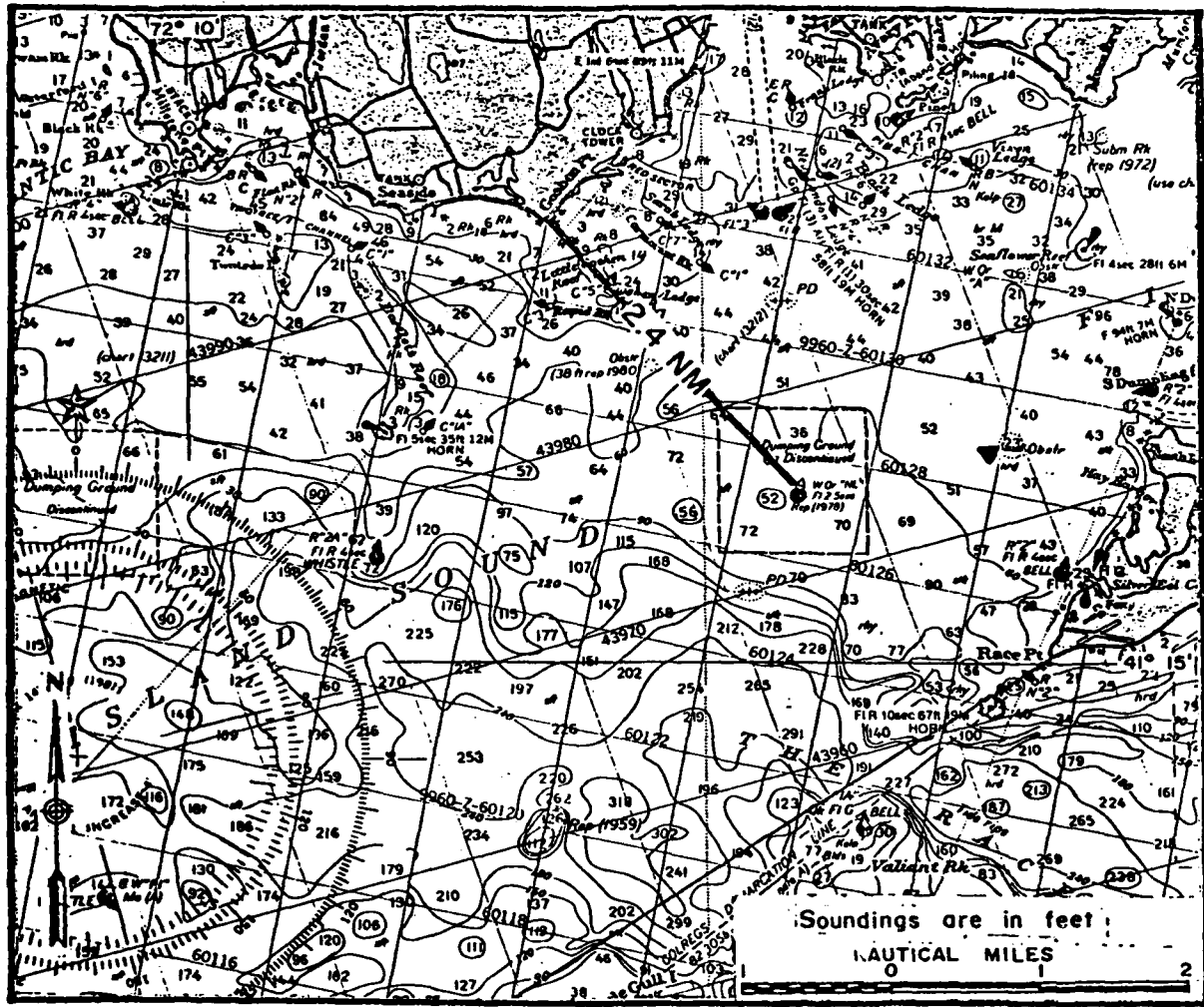
▼ SAMPLING SITE FOR REFERENCE SEDIMENT



## CENTRAL LONG ISLAND SOUND

**NED 3: CENTRAL LONG ISLAND SOUND**      N.O.S. CHART: 12354  
**DEPTH RANGE: 49-75 FEET MLW**      DATE: 19 APRIL 1980  
**CENTER COORDINATES: 41°-08.95'N, 72°-52.85'W**  
**DESCRIPTION:** THIS SITE IS 2 NAUTICAL MILES LONG BY 1 NAUTICAL MILE WIDE WITH THE MAJOR AXIS RUNNING TRUE EAST-WEST AND CENTER AT 41°-08.95'N LATITUDE AND 72°-52.85'W LONGITUDE. FROM THE CENTER, SOUTHWEST LEDGE LIGHT BEARS TRUE 345° AT 10,750 YARDS AND TOWNSHEND LEDGE LIGHTED GONG BUOY No. "10-A" BEARS TRUE 13° AT 7,400 YARDS. THIS SITE IS APPROXIMATELY 5.6 NAUTICAL MILES OFF SOUTH END POINT, EAST HAVEN.

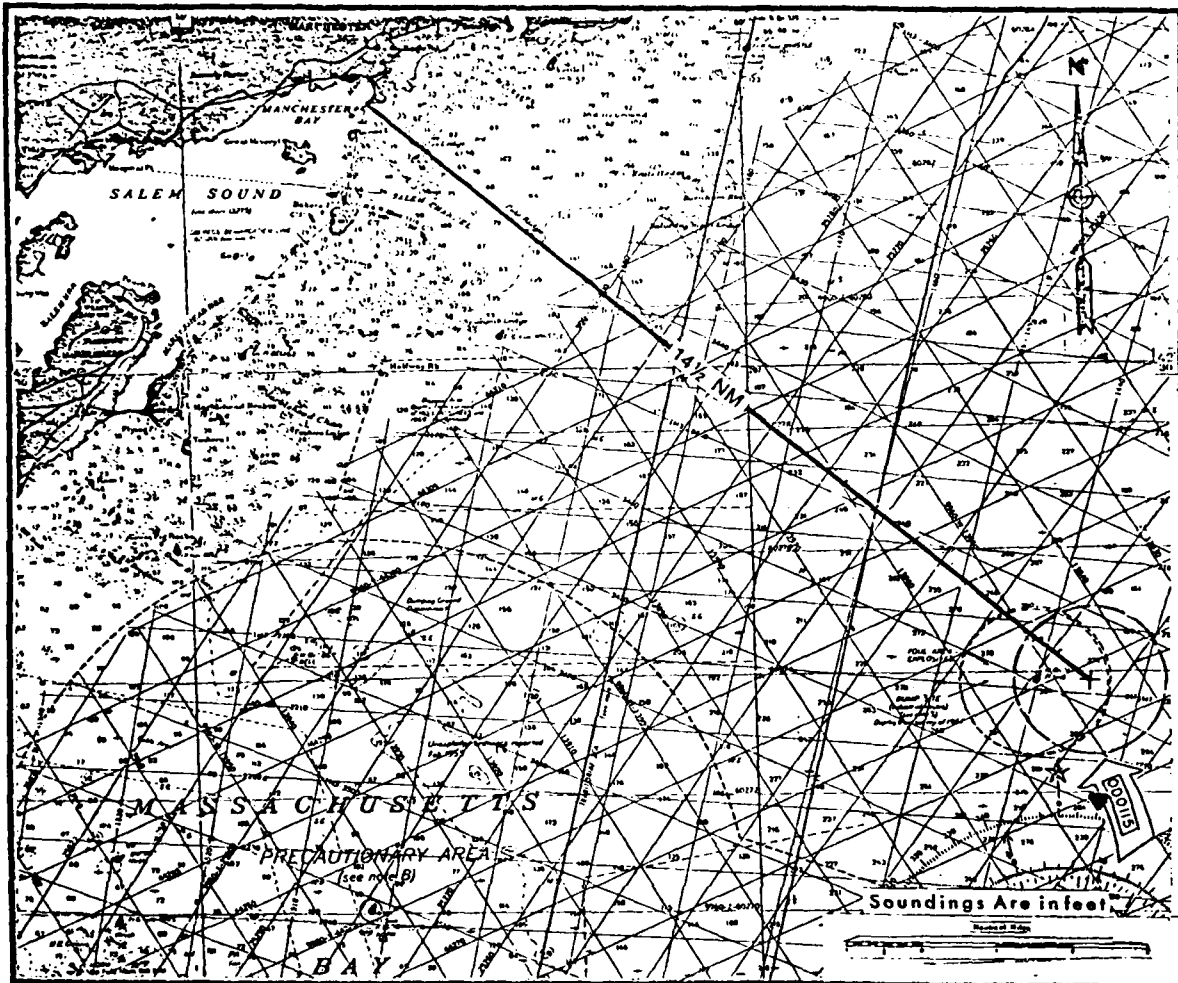
▲  
 SAMPLING SITE  
 FOR REFERENCE  
 SEDIMENT



## NEW LONDON, CT.

**NED 6: NEW LONDON**      **N.O.S. CHART: 13205**  
**DEPTH RANGE: 36 TO 72 FT MLW**      **DATE: 21 FEBRUARY 1981**  
**CENTER COORDINATES: 41°-16.1'N, 72°-04.6'W**  
**DESCRIPTION: THIS SITE IS 1 NAUTICAL MILE SQUARE WITH CENTER**  
**AT 41°-16.1'N, 72°-04.6'W AND SIDES RUNNING TRUE NORTH-SOUTH,**  
**EAST-WEST, FROM THE CENTER, BARTLETT REEF LIGHTED HORN BEARS**  
**TRUE 276° AT 5,600 YARDS, AND BLACK LEDGE LIGHTED BUOY N<sup>o</sup> 2**  
**BEARS TRUE 130° AT 4,000 YARDS.**

▼ **SAMPLING SITE**  
**FOR REFERENCE**  
**SEDIMENT**

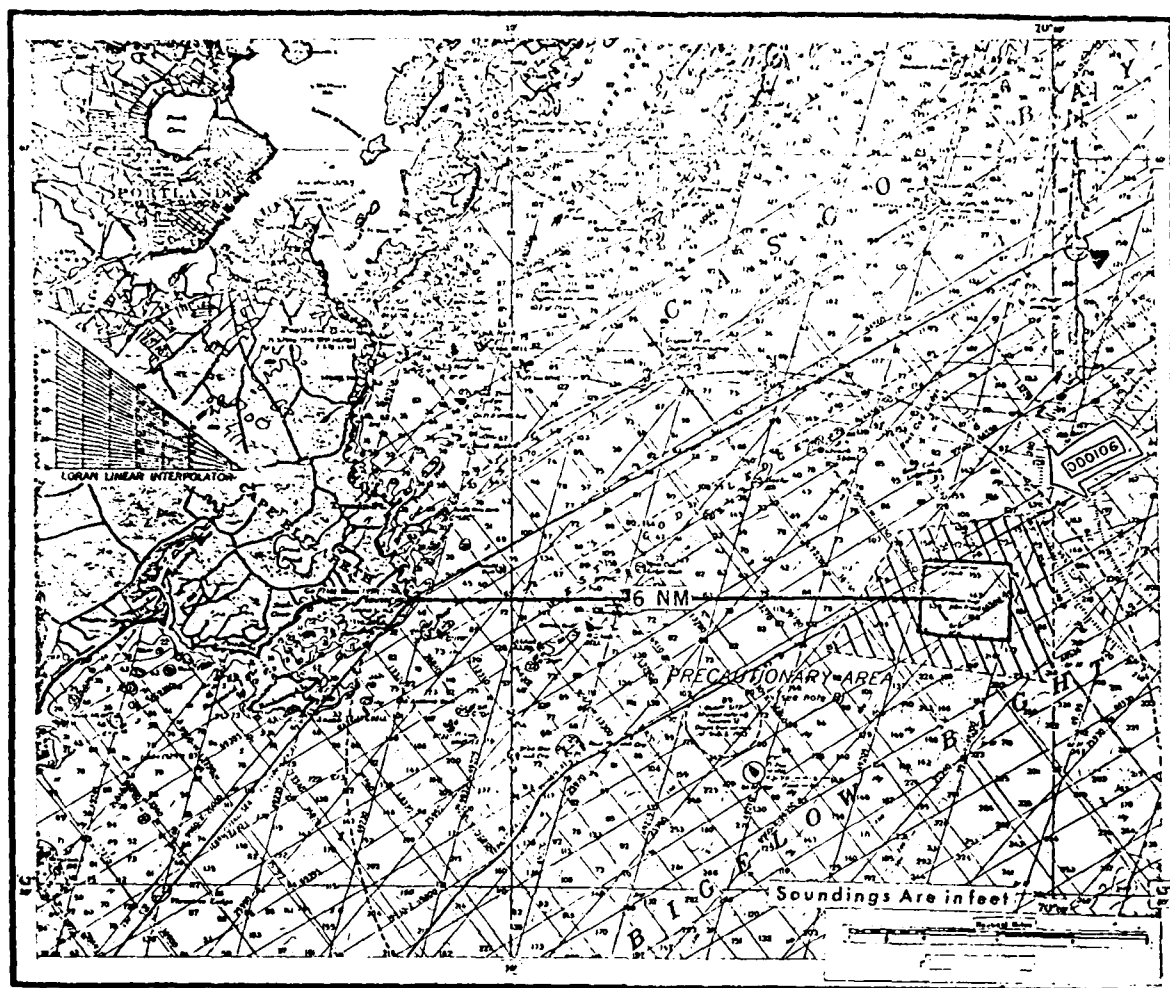


## FOUL AREA, MASS. BAY

EPA OD0115: FOUL AREA  
 DEPTH RANGE: 159 TO 304 FEET MLW  
 CENTER COORDINATES: 42°-25.7'N, 70°-34.0'W  
 DESCRIPTION: THIS EPA APPROVED INTERIM SITE IS A CIRCULAR AREA WITH A DIAMETER OF 2 NAUTICAL MILES AND CENTER AT 42°-25.7'N, 70°-34.0'W. FROM THE CENTER, THE MARBLEHEAD TOWER BEARS TRUE 282° AT 24,300 YARDS AND BAKERS ISLAND HORN BEARS TRUE 300° AT 24,300 YARDS.

N.O.S. CHART: 13267  
 DATE: 20 DECEMBER 1980

▼  
 SAMPLING SITE  
 FOR REFERENCE  
 SEDIMENT



## PORTLAND, GULF OF MAINE

EPA 000106 PORTLAND

DEPTH RANGE: 135 TO 226 FEET MLW

CENTER COORDINATES:  $43^{\circ}-34.1'N$ ,  $70^{\circ}-01.8'W$

DESCRIPTION: THIS EPA APPROVED INTERIM SITE IS 1 NAUTICAL MILE SQUARE WITH CENTER AT  $43^{\circ}-34.1'N$ ,  $70^{\circ}-01.8'W$  AND SIDES RUNNING TRUE NORTH-SOUTH, EAST-WEST. FROM THE CENTER, HALFWAY ROCK HORN BEARS TRUE  $357^{\circ}$  AT 10,800 YARDS AND WEST COD LEDGE LIGHTED WHISTLE BUOY R "2" BEARS TRUE  $269^{\circ}$  AT 9,100 YARDS.

N.O.S. CHART: 13288

DATE: 8 DECEMBER 1979

▼ SAMPLING SITE  
FOR REFERENCE  
SEDIMENT

Dr. Gary Chapman  
U.S. Environmental Protection Agency  
Corvallis, OR

## Suggested Dredge Material Assessment Procedures<sup>1</sup>

by Gary Chapman

Corvallis Environmental Research Laboratory

Assessment of the contamination level of sediment has been conducted using either chemical or biological procedures. Chemical analysis of the bulk concentration of potential pollutants has been conducted for a number of years, and various chemical criteria have been proposed for evaluating the level of contamination or the acceptability of dredged sediment for inwater disposal. There are several major drawbacks to this type of sediment criteria. First, they require sophisticated chemical analytical equipment. Second, they are not available for all chemicals of concern, so that acceptable levels for some chemicals cannot be considered a priori evidence that no other chemicals are present in harmful quantities. Third, the existing chemical criteria for sediments have, in many cases, been established on weak scientific premises and often do not prove reliable as predictors for biological effect levels.

Bioassays of sediments provide information on the probable biological effects of sediments, although extrapolation from bioassay to field is an uncertain step. The uncertainty is largely a function of the reality of the test exposure and the representativeness of the bioassay organisms(s). Bioassays can be used either as predictors of effects in the field or as simple pass-fail tests based upon agreed upon protocols. Development of standard bioassessment protocols

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<sup>1</sup> This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.



for sediments is not a mature discipline although considerable progress has been achieved over the past several years.

Among the possible effects of sediment contamination, the three most obvious are: lethality to aquatic organisms; sublethal effects on aquatic organisms; and potential health effects to persons ingesting water or contaminated food. Among the lethal and sublethal effects on aquatic organisms, there are two classifications. The first includes all effects normally measured in routine toxicity bioassays; e.g., effects on survival, growth, and reproduction; the second includes a group of effects not normally detected in routine bioassay procedures; e.g., disease susceptibility, lesions, oncogenicity, and carcinogenicity.

Routine toxic effects can be measured by any of a number of existing bioassay procedures. Selection of procedures depends upon whether the purpose of the test array is to predict the probable biological effect at the site of contamination or disposal, or whether it is to function as a simple pass-fail bioassessment procedure. In reality, the cost of predictive bioassessment arrays is usually prohibitive. As a result, in most instances what is needed is a sensitive yet simple pass-fail test that can be used to trigger a decision process. Depending upon the magnitude of the project cost or the public concern, the results of the initial test may inevitably lead to a second tier of bioassessment regardless of the outcome of the initial tier. This is because "pass" doesn't prove "it's safe" and "fail" doesn't prove "it will really have a significant effect." One value of a standard bioassessment protocol is to minimize the number of instances that will escalate to successive test tiers.

The nontypical effects to aquatic organisms, especially those effects that may represent human health concerns (read carcinogenicity), are not readily tested in reliable, standard ways. All existing protocols that in some way measure disease, genotoxicity, tumor induction, etc. have several problems. None that I know of have any reasonable degree of field validation. Some may produce a large proportion of false positive results with respect to the field (e.g., correlation of Ames test results to tumors in aquatic organisms). Others may require lengthy exposure or post-exposure periods prior to the development of sediment induced effects.

Evidence of tumors, lesions etc. in organisms at the dredging site can be considered strong evidence of contaminated sediments. Absence of externally obvious histopathology cannot, however, be relied upon as a clean bill of health, so that microscopic histopathology would be required for reasonably definitive testing.

Chemical analysis of sediments for known or suspect carcinogenic chemicals or classes of chemicals might be used to trigger further laboratory tests or field studies. Selection of a roster of such chemicals might prove difficult and if the list were relatively complete most sediments could be found to contain at least a trace amount of one-or-more listed chemicals. What combination of chemical numbers or concentrations would be used to trigger a further tier of testing? If a very sensitive trigger is used then perhaps Ames Tests or similar protocols would be called for so frequently that they may as well be conducted routinely.

Finally, if a sediment does fail a mutagenicity (or similar) screen what further decisions does that imply? Should the sediment be left in place, disposed of in water, or disposed of on land? Depending on the test protocols selected and the results observed, any number of outcomes may occur. With a triad of chemical analysis, mutagenicity tests, and a histopathology survey of site organisms, at least eight outcomes are possible (Table 1). Decision making with the complete triad could be complex, and examination of Table 1 suggests possible pitfalls if only one or two protocols are followed.

Table 1. Possible outcomes of tests to ascertain risk of nontypical toxic effects of sediment contaminants to aquatic organisms or their consumers.

Test	Possible Outcomes							
	1	2	3	4	5	6	7	8
Chemical presence <sup>A</sup>	-	+	-	-	+	+	-	+
Mutagenicity <sup>A</sup>	-	-	+	-	+	-	+	+
Histopathology <sup>B</sup>	-	-	-	+	-	+	+	+

<sup>A</sup>Sample specific.

<sup>B</sup>Integrates broadly over time and space.

- 1 - No apparent problem (except possible toxicity or bioaccumulation).
- 2 - Exposure conditions not sufficient for effect.
- 3 - Unknown mutagen present, no discernible site effect.
- 4 - Something affecting organisms at site, perhaps not sediment.
- 5 - Potential cause and effect but not discerned in site organisms, possible risk to consumer if bioaccumulated in food.
- 6 - Possible false negative mutagenicity test or combination of cases 2 and 4.
- 7 - Unknown mutagen may be related to effect seen in site organisms.
- 8 - Probable cause-effect link between sediment contaminants and effects.

The final decision of which protocol(s) to adopt depends on cost, relative sensitivity, and the details of the regulatory instrument being invoked. Following study of Table 1 I am of the opinion that a mutagenicity type test would be the best single protocol, although the acceptance of the results should be contingent upon a reasonable amount of validation showing an acceptable proportion of false positives and very few false negatives.

Finally, effects on human health, primarily through the ingestion of contaminated fish or shellfish is an area that cannot be ignored. However, it has been my position that procedures for determining safe levels of tissue "contamination" are difficult to establish, and that in the absence of FDA action levels, oral toxicity data, or dietary intake maxima established by health/regulatory agencies, there is no basis (excepting lengthy mammalian studies) upon which to judge the acceptability to consumers of a tissue level for any chemical. The mere presence of a chemical in sediments is an almost certain indication that the same chemical will be found in organisms having intimate contact with the sediment or in organisms having a significant sediment-based food source.

Bioconcentration/bioaccumulation to tissue levels greater than sediment levels is not necessary for unacceptable tissue levels nor is significant bioconcentration/bioaccumulation a sign of unacceptable tissue levels. Acceptability of tissue concentrations to consumers is a function of the concentration of the chemical and its inherent toxicity, and has little known correspondence with the mere fact or degree of bioconcentration/bioaccumulation.

For those chemicals for which some type of regulatory tissue levels exist, I would simply measure their level in sediment. Because nearly all such chemicals are neutral organics, a reasonable extrapolation should be possible to estimate tissue levels of organisms that are in thermodynamic equilibrium with the sediment. In most instances, this will provide a worst case scenario and where it does not, the answer should be acceptably close. Alternatively, tissue levels in organisms from the dredging site could be measured, with the assumption that they will represent values that would result at the disposal site. Sampling, extraction, and data analysis may be more complicated for the biological samples than for the sediment samples.

#### Recommendations

Toxicity to aquatic organisms. Conduct acute and chronic toxicity tests with a sensitive organism. I suggest elutriate and solid phase tests with Daphnia sp. Tests starting the 5-day old daphnids and continuing for 10 days provide both acute and chronic toxicity data.

Disease, tumors, etc. Conduct Ames test on sediment extracts. Alternatively, or secondarily, conduct a field survey to see if significant histopathological problems exist at the site of dredging.

Tissue contamination. Measure regulatable chemicals in sediment and extrapolate to maximum tissue levels using existing models.

Daphnia bioassay. The Daphnia bioassay is recommended because of its relative ease, sensitivity, and standardization. Daphnia are generally more sensitive

than other more sediment-associated organisms (e.g., worms, amphipods, and insect larvae) and do feed at the sediment water interface in bioassays which call for a sediment phase. Starting with 5-day-old daphnids makes recovery and observation of daphnids much easier during the first few days of the test, and use of D. magna especially makes it easier to count offspring at the termination of the test. D. pulex usually are preferable to D. magna in waters with total hardness below 50 mg/L as CaCO<sub>3</sub>. Ceriodaphnia tests are also good, but may be difficult in tests with sediments present (because of the small size of the young.)

Conducting both elutriate and solid phase bioassays provides more data. The elutriate test allows for dilution and dilution allows a measure of how toxic a contaminated sediment really is. The solid phase bioassay allows for cases in which sediment presence significantly increases or decreases toxicity from that seen in the elutriate bioassays. Tests should include a control sediment that supports survival and reproduction of the daphnids. For management decisions, bioassay of disposal site sediments should also be included to provide information on the relative hazard represented by both the dredged sediment and the sediment at the disposal site. (The latter could be more contaminated than the former.) Disposal site water should be used with all sediments (except one control sediment test with control water) in order to separate sediment effects from water effects and to provide a realistic test matrix.

Selection of a sediment sampling scheme is important and should include consideration of both vertical and horizontal variation in sediment contamination as appropriate for both the dredging and disposal sites.

## BIOLOGICAL METHODS FOR DETERMINING TOXICITY OF CONTAMINATED FRESHWATER SEDIMENTS TO INVERTEBRATES

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**Abstract** — Methods are presented for using *Daphnia magna*, *Hyalella azteca*, *Gammarus lacustris*, *Chironomus tentans* and *Hexagenia limbata* to screen freshwater sediments for acute and chronic toxicity, bioaccumulation potential and *in situ* toxicity. The 48-h *Daphnia* tests are recommended as inexpensive, uncomplicated and sensitive acute methods. *Hyalella* and *Chironomus* are the recommended benthic test organisms, as they are easy to rear and test, they remain in intimate contact with the sediment and they exhibit high control survival. Verification studies (published elsewhere) evaluating the recommended methods and organisms are briefly summarized.

**Keywords** — Sediment bioassay methods    Acute toxicity tests    Chronic toxicity tests  
Freshwater invertebrates

### INTRODUCTION

This article describes rearing procedures, acute and chronic testing methods and bioaccumulation and *in situ* methods for estimating the potential impact of contaminated sediments on aquatic life. The specific tests presented here to screen freshwater sediments for toxicity use five freshwater invertebrates: *Daphnia magna* (cladoceran), *Hyalella azteca* (amphipod), *Chironomus tentans* (midge), *Gammarus lacustris* (amphipod) and *Hexagenia limbata* (mayfly).

Three types of acute tests are described: (a) a liquid phase elutriate test using

*Daphnia*; (b) a solid phase sediment and water beaker test using one or several of the five organisms; and (c) a sediment and water Prater-Anderson type test using *Daphnia* and *Hexagenia*.

Four chronic tests are described: (a) a larval survival and growth test with *Chironomus*; (b) an adult-emergence test with *Chironomus*; (c) a *Hyalella* partial life cycle test; and (d) a *Daphnia* life cycle test.

Laboratory bioconcentration test methods are described, and an *in situ* test is discussed that may be used in the field for toxicity studies, laboratory-field comparisons or field bioconcentration determinations. A brief summary of verification tests (published elsewhere) conducted to evaluate the proposed methods is presented, along with a literature review and criteria to be used for test selection.

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Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Testing procedures approximate worst-case conditions, as potential toxic materials from the sediment will be retained in the water in the beakers of both the elutriate test, containing only dissolved materials, and the tests containing water and solid phase sediment. In the solid phase sediment and water tests, toxicants bound to particulates may also be available to the animals when they feed on or come in contact with the sediment.

The acute procedures recommended here are relatively inexpensive first-step screening processes to determine short-term acute toxic effects of contaminated sediments; they cannot indicate whether or not the sediments are free of toxic effects. Longer-term exposure, chronic life cycle tests, bioconcentration tests, *in situ* assays and assessment of invertebrate populations in the field are needed to show that the sediments are a hazard to freshwater aquatic life.

#### METHODS

##### *Rearing of animals*

All culture and testing procedures are done using a photoperiod of 16 h light:8 h dark, with all temperatures at 20°C, except for *Gammarus*, which are reared and tested at 16°C.

*D. magna*. *Daphnia* are reared in 4-liter jars containing 3 liters of water maintained at a hardness of >100 mg/L as CaCO<sub>3</sub> (a lower hardness reduced survival and young production; 200 mg/L is recommended). Only 50 adults are contained in each jar, to ensure adequate oxygen concentration without aeration. Eight jars are maintained in the culture, with two jars containing new age groups added each week, discarding those >30 d old. The water is changed two or three times each week, with some food being added daily. The water is poured through a 1.5-mm mesh screen (10 × 15 cm) to retain adults, and they are transferred to a jar of fresh water and new food added.

The young to be used for testing or starting new cultures are retained by a fine screen (<0.5-mm mesh, 11 × 16 cm, with up-turned edges) placed below the 1.5-mm mesh screen. At each fresh water change *Daphnia* are fed algae (*Selenastrum capricornutum*) and a fish food (Oregon Moist Pellet®) and yeast mixture [1] at a dry solids concentration of 2.0 mg/L algae and 5 mg/L fish food-yeast. On days when water is not changed they are fed 0.5 mg/L algae.

*H. azteca* and *G. lacustris*. *Hyalella* are the preferred species as they reproduce continually and grow rapidly, and burrow in the sediment when disturbed. *Hyalella* and *Gammarus* should be reared in 10- or 20-liter aquaria under flowing-water conditions if possible. *Gammarus* require several weeks of a short light period to initiate reproduction (e.g., 10 h light:14 h dark at 10°C). If static conditions are used, the water should be partially (e.g., 30%) changed once a week and gently aerated. It is unnecessary to maintain carefully controlled feeding regimens for *Hyalella* and *Gammarus*, as is done for *Daphnia*. Dried maple, alder, birch or poplar leaves (presoaked for several days) are used as the primary substrate and food for *Hyalella* and *Gammarus*. Rabbit food pellets, Cerophyl®, fish food such as Tetra® Conditioning Food, frozen or newly hatched brine shrimp or heat-killed young *Daphnia* serve as good food if used sparingly. To clean the rearing chamber or reduce populations of animals when they become abundant, transfer half of the leaf substrate (containing some animals) to a sorting tray, discard the remainder of the old chamber contents and return the leaf substrate and animals to the chamber. *Hyalella* will need to be thinned periodically because the population expands rapidly.

*C. tentans*. Midge cultures in flow-through 10-liter aquaria have been successful [2,3], but static test containers can be used if they are aerated and the water is



changed partially each week. Two egg masses are placed in an aquarium covered with an adult flight cage [3], with water flowing through the aquarium (50–100 ml/min) if possible. When eggs hatch (at 3 d), and once again 3 d later, 100 mg (0.25 ml dry volume) Cerophyl is placed in the aquaria for food and substrate (the larvae bind the Cerophyl together with silk to form tubes in which they live). The Cerophyl is mixed with water and then poured into the rearing chamber; several aquaria can be fed from a slurry prepared in one beaker. Finely powdered Tetra (10% of Cerophyl volume) can be added for a more balanced diet, especially in static containers where there is no nutrient input from the flowing water source. Two hundred milligrams (0.5 ml dry volume) Cerophyl (plus 10% Tetra) is added to the container twice a week thereafter; more may be needed with older larvae. Too much food will cause a fungal mat to develop that will kill young midges if it is not broken up; too little food will cause the midges to scrape bare spots on the container bottom between larval tubes. They will leave their burrows if insufficient food is available, so maintain a light "dusting" of fresh Cerophyl on the bottom of the container. The buildup of undigestible material over the 30 d of the life cycle provides the large mass of tube-building material required by the third- and fourth-instar larvae.

Second-instar larvae for use in testing can be collected from the aquarium 10 d after egg hatch (at 20°C). If several containers are set up [3] and eggs are added to each new container at 3-d intervals, larvae for tests will always be available and adults and eggs will be available for starting new cultures.

*H. limbata*. This species requires a substrate, but unlike the midges, they do not construct it from particles such as Cerophyl. They require a fine-textured high-organic sediment into which they can burrow and which will retain the burrow integrity (e.g., sand will collapse). The ideal substrate would be from the animal's native area. If

eggs or young animals are received from a vendor (e.g., bait dealer or research laboratory), then clean sediment (free of predators) should be collected and used as substrate. It is difficult to determine how much to feed the larvae, especially when different-sized animals are in the culture. However, the addition of 1% by volume of an 80:20 mix of Cerophyl and Tetra to the chamber [e.g., 20 ml (8 g) food to 2,000 ml sediment in a 20-liter aquarium] will provide sufficient food to initiate the culture. Weekly additions of the same amount should maintain the culture, unless fungus appears on the sediment surface, which indicates that too much food is being added.

A flow-through rearing facility [3] should be used, because the water will remain clear and the animals and burrows (or fungus if overfed) can be easily observed. If static tanks are used (with concomitant turbid water of near-zero visibility), gentle aeration and partial (e.g., 20%) weekly water change are required. Five centimeters of substrate is adequate for culture of *Hexagenia*. Brushing or stirring the sediment surface is useful for circulating the food across the mud surface and for determining numbers of animals in the holding tank in flow-through systems, because the larvae will rapidly form new burrows, which facilitates counting. Five hundred newly hatched or 100 older larvae (up to 15 mm in length) can easily be maintained in a 40-liter aquarium.

#### Acute test methods

Three acute test methods are described: (a) the liquid phase elutriate test in which water is mixed with contaminated sediment (1:4, v/v, mix of sediment and water) and then settled and centrifuged—the water is used to test *Daphnia* by exposing them to dissolved materials extracted from the sediment; (b) the solid phase sediment and water test in which sediment and water (1:4, v/v, ratio of sediment and water), together in the test chamber, are used to expose the test species to both dissolved and bound materials in the sediments; and (c) the solid phase

sediment and water Prater-Anderson test (1:9.5, v/v) in which *Hexagenia* are placed in the sediment and *Daphnia* suspended in a cage in the recirculating water column.

Appropriate statistical analyses to determine significant differences in animal survival between test sediments and control sediments are required. Chi-square analysis can be used to test for differences between control and test survival at any desired level of significance.

Routine chemical analyses such as dissolved oxygen, pH, total hardness and total alkalinity should be conducted to assure that the water is satisfactory for the animals. Other analyses such as particle size, organic content, heavy metals, ammonia, organics, etc., may be required to better characterize the sediment.

Sediment samples are collected and held at 4°C until used (preferably <2 weeks of storage). They should be thoroughly mixed and screened through one or more standard sieve screens to remove large particles and endemic animals, especially predators. An uncontaminated control sediment with similar particle size and organic content should be collected and treated similarly.

To obtain <24-h-old *Daphnia* for testing, adults and young in rearing jars (screened the day before to remove young) are poured onto a 10 cm × 15 cm screen of 1.5-mm mesh to retain adults only, which are returned to the rearing jars. A 11 cm × 16 cm fine screen (<0.5 mm mesh) with upturned edges is placed under the 1.5-mm mesh screen to retain young, which are then transferred to other containers. *Daphnia* are counted and transferred to test containers with a 5 mm inner diameter glass pipette. For solid phase testing, young are fed and reared for 5 d before testing so that they will be large enough to recover at the termination of the test.

Transferring the other animals from rearing containers to test beakers is relatively easy. *Hyalella* and *Gammarus* are separated from the leaf material in their rearing chamber by scooping up the leaves (containing

animals) and placing them on a 5 to 10-mm mesh plastic screen placed over a white porcelain pan containing 2 cm water. By sprinkling water on the leaves while turning and separating them, the animals are washed from the leaves and drop through the screen into the pan and are immediately available for use. *Hexagenia* can be screened and rinsed from the rearing substrate with a 2-mm mesh screen and rinsed into the pan. The midges can be pipetted directly from the water containing them and their substrate (poured from the rearing chamber into the pan) because they swim free of their burrows when disturbed by water turbulence. *Hyalella*, *Chironomus* and young *Gammarus* can be transferred from the pan to test chambers with a 7 mm inner diameter glass pipette. *Hexagenia* and larger *Gammarus* may be transferred with a spoon-shaped piece of screen.

*Liquid phase elutriate test with D. magna.*

This procedure is adapted from the methods described in the joint U.S. Environmental Protection Agency (EPA)-U.S. Army Corps of Engineers manual [4] for preparation of liquid phase marine dredge samples. *Daphnia* are exposed for 48 h to centrifuged water samples obtained from a sediment-water slurry. The sediment is mixed with clean dilution water in a volumetric sediment-to-water ratio of 1:4 and placed in a closed container (e.g., 350 ml sediment/1,400 ml water in 2-liter bottle) and mixed vigorously for 30 min. The samples are then allowed to settle overnight. The overlying water is siphoned off and centrifuged at 10,000 rpm for 15 min. (Centrifugation has been shown to be more efficient than 0.45- $\mu$ m filtering in removing undissolved components from the water [W. L. Griffis, U.S. EPA, Corvallis, OR, personal communication].) The water (200 ml/beaker) is then transferred directly to three 250-ml beakers. They are gently aerated with glass-tipped plastic air lines, with the tip 1 cm below the water surface.

Ten *Daphnia* (<24 h old) are placed in each of the three beakers containing the elutriate water to be tested. If the sample kills all animals in 48 h, the centrifugate can be diluted or a new sample can be prepared and diluted several times to determine relative toxicity of the sediment sample.

*Solid phase sediment and water beaker test.* These tests, conducted in aerated 1,000-ml beakers [3], expose *Daphnia* for 48 h and *Hyalella*, *Chironomus*, *Gammarus* and *Hexagenia* for 10 d, at which time survivors are screened from the water and sediment and counted, using survival as the criterion for toxicity.

Sediment (200 ml) is placed in each of three replicate 1,000-ml glass beakers. After addition of the sediment, 800 ml of dilution water is gently poured into each beaker, bringing the total contents to 1,000 ml. Beakers should be left unaerated overnight to reduce turbidity and to allow more time for water-sediment contact before animals are placed in the beakers. The water in the beakers should be aerated for 30 min before test animals are added, using glass-tipped plastic air lines from an air source. Gentle aeration with the tip 3 cm below the water surface is used to aerate the water, avoiding any disturbance of the sediment that would create unnecessary turbidity.

Fifteen test animals are then placed in each beaker: Of those animals studied, 5-d-old *Daphnia*, juvenile *Hyalella* and 2nd-instar *Chironomus* larvae were found to be the easiest to rear and test in the laboratory. *Daphnia* and *Chironomus* can be tested together in the same beaker. Ten early-instar *Hexagenia* (<10 mm long) or juvenile *Gammarus* (<7 mm long) may also be used in each beaker. Cannibalism and high control mortality results from using larger *Gammarus* and *Hexagenia* in the 1,000-ml beakers. However, larger containers with more sediment, such as 4-liter jars, can be used (10 animals/jar) if small animals are not available. At the end of the test the sediment is screened (0.5–2-mm mesh depending on

animal size) to collect and count the live and dead animals remaining in the sediment. Good *Daphnia* recovery after 48 h is obtained by pouring water and fine suspended sediment, but not the bulk of the sediment, through the 10 cm × 15 cm (0.5-mm mesh) screen. If *Daphnia* and *Chironomus* are tested together, the water and fine sediment can be returned to the beaker with *Chironomus* for completion of the 10-d exposure. The *Daphnia* are then gently rinsed from the screen and transferred to clean water for counting.

*Solid phase sediment and water Prater-Anderson test.* Use of this system [5,6] containing both *Daphnia* and *Hexagenia* (20 and 5 per replicate, respectively) will generally give results comparable to those of the beaker test [7]. However, this test is more time-consuming to construct, calibrate and use for bioassays, so the beaker tests are preferable. In beaker tests, *Daphnia* have direct contact with the sediment, whereas with the Prater-Anderson device, they are retained in a cage in the water column. The *Daphnia* (<24 hr old) in the Prater-Anderson system are exposed to dissolved and particulate materials due to *Hexagenia* activity. The Prater-Anderson system does have larger volumes of sediment and water, as well as a larger water/sediment ratio (9.5:1), so that larger *Hexagenia* can be used and more water samples can be removed to monitor chemical conditions.

#### *Chronic tests*

*Chironomus adult emergence test.* This 25-d test is adapted from the midge life cycle test found in *Standard Methods* [8], and is a relatively inexpensive procedure designed to encompass most of the life cycle of this animal. The test is started with 10-d-old (20°C) second-instar larvae, and the endpoint is a count of emerged adults.

The sediment to be tested is placed in containers, such as 20-liter aquaria or 4-liter jars, and covered by screening to retain adults. The sediment layer should be 2 to 3

cm deep, overlaid with 15 cm of gently aerated water. Distilled water is used to replace water lost by evaporation. One hundred larvae are added to the sediment at the start of the test. A food mixture of 600 mg Cerophyl (1.5 ml dry volume) and 100 mg (0.3 ml) finely crushed Tetra flakes should be mixed with distilled water in a small beaker and fed to the animals at the start of the test and again on day 8. On day 14 they should be fed 800 mg (2.0 ml) Cerophyl and 100 mg (0.3 ml) Tetra, and on day 18 they should be fed 1,000 mg (2.5 ml) Cerophyl and 100 mg (0.3 ml) Tetra. If larvae are dying and not consuming the food, fungus will appear, indicating that less food should be added. Adults should begin emerging after 20 d; the test should be continued for another 5 d to count all adults emerging and to observe for delayed development. A small vacuum pump with a 10-mm diameter plastic line running through an Erlenmeyer flask trap is used to collect adults and make daily counts of those emerging (the screen cover is slowly and gently lifted off the container, and the adults are vacuumed from the screen and the inside walls of the container).

*Chironomus larval survival and growth test.* If the larvae need to be retained for bioconcentration studies, follow the same initial procedures used for the adult emergence test, but use larval survival and growth (length and weight) after 15 d as the endpoints of the chronic test. The sediment can be screened to collect the larvae, which are placed in water only overnight to clear the gut. They are then killed with warm water, blotted dry, weighed (mg) and measured (mm) and frozen for later tissue analysis.

*D. magna life cycle test.* This relatively inexpensive test is started with 5-d-old *Daphnia* and exposes them for 10 d, through maturation and release of young (three broods), at which time the test is terminated and adults and young are counted. Total number of surviving adults and young,

compared to controls, is the criterion for establishing sediment toxicity.

Four-liter jars, holding 2.5 liters of water plus 500 ml of test sediment, can be used as test containers. The test should be started by adding 20 5-d-old *Daphnia* to each jar along with food at the rate of 2 mg/L solids (1.0 mg/L algae such as *Selenastrum*, plus 1 mg/L solids from the fish food-yeast mixture). They should be fed every other day until the end of the 10-d test. The containers should be gently aerated with a glass-tipped plastic air line, with the tip 4 cm below the water surface (sufficient to aerate the water without disturbing the sediment). At the end of the test, water and fine suspended solids, but not the bulk of the sediment, is poured through the 0.5-mm mesh (10 cm × 15 cm) screen to retain surviving adults and young *Daphnia*. They are then gently rinsed from the screen and transferred to clean water for counting.

*Hyalella partial life cycle test.* Use the methods described for the *Hyalella* bioconcentration test. Endpoints for the 28-d chronic exposure are the number of adults and young surviving. Sediment should be screened through a 1.0-mm mesh standard sieve before testing, if possible (may be diluted and mixed 1:1 with test water to facilitate screening), as particles should be smaller than the young animals so they can be effectively screened (0.5-mm mesh) and recovered from the sediment at the end of the test.

#### *Bioconcentration tests*

These methods are designed to expose *Hyalella*, *Gammarus* or *Hexagenia* for 28 d, and *Chironomus* for 15 d, after which the animals are removed for tissue analysis of the toxicant(s) being monitored.

The test containers are 20-liter aquaria with 2 to 3 cm of test sediment on the bottom overlaid with 15 cm water. If less sediment is available for testing, 4-liter jars can be used, but proportionally fewer animals and less food should be used. Sediment

should be screened so the particles are smaller than the animals to be tested, if possible, so that the animals can be more easily screened from the sediment at the end of the test.

The test, using 20-liter aquaria, is started with 100 *Hyaella* or juvenile *Gammarus* (<7 mm long), second-instar *Chironomus* or early-instar (10–15 mm) *Hexagenia*. If larger *Gammarus* or *Hexagenia* are used, 25 to 50 animals should be used. They should be placed in each of two or more replicate aquaria and exposed for 28 d (15 d for *Chironomus* larvae). At the end of the test, animals should be screened from the test water and sediment, placed in clean water overnight to clear the gut and then frozen for later analysis.

Food for the animals, in addition to what they obtain from the sediment sample, should be Cerophyl and Tetra for *Chironomus* and *Hexagenia*, and rabbit food pellets for *Hyaella* and *Gammarus*. The Cerophyl–Tetra food mixture [600 mg Cerophyl (1.5 ml dry volume) and 100 mg (0.3 ml) powdered Tetra] should be mixed with 100 ml distilled water in a small beaker and dispersed over the water surface. Give 200 mg (0.5 ml dry volume) rabbit food (soaked and dispersed in 100 ml distilled water) to *Hyaella* and 600 mg (1.5 ml) to *Gammarus* at each feeding. Animals should be fed twice a week during the exposure period. If mortalities occur, feeding should be reduced proportionally. All tanks should be aerated and water lost to evaporation should be replaced with distilled water.

#### *In situ* test

The basic concept of an *in situ* bioassay for benthic invertebrates is to expose the test animals at the field site, without disturbing the contaminated sediment, and determine percent survival. With fish tests for water-only exposure the process is simple: A cage is hung in the water column or anchored to the bottom and the fish are transferred to the cage and exposed for 96 h or longer. Testing invertebrates in the undisturbed sediment

poses more problems, such as predation and recovery of the animals.

A 9 cm × 20 cm cylindrical stainless steel screen (1.5-mm mesh) cage is used to contain the test animals. The cage is closed with a petri dish at one end, with another petri dish placed over the open end after the test animals are introduced. Twenty adult *Hyaella*, juvenile (7–10 mm) *Gammarus*, early-instar (10–15 mm) *Hexagenia* or third-instar *Chironomus* should be used in each cage. Only 10 larger *Gammarus* or *Hexagenia* should be used, and all should be of the same length. If *Chironomus* are used, the screen should be 0.75-mm mesh rather than 1.5-mm, but this mesh can only be used with sediments of very small particle size. The animals are not fed during the exposure. An uncontaminated control site with similar sediment conditions should be selected to expose and recover control test animals. The animals are acclimated to site temperature, and are carried to the test site in a container with water. The cage is partially submerged in water at the test site, the animals gently added and the cover fitted securely. One-fourth of the cage, if possible, is then gently forced lengthwise into the sediment and supported with stakes. The animals are exposed for 96 h using at least two replicate cages. The cage is then removed from the sediment (with the cover still in place), gently washed free of sediment and the enclosed test animals removed and counted.

#### TEST METHODS EVALUATION STUDIES

A series of tests was conducted at this laboratory, using field-contaminated and artificially spiked sediments, to develop and validate the proposed methods. The results of these evaluation studies (published elsewhere) are briefly summarized here.

Malueg et al. [7] used the test apparatus developed by Prater and Anderson [5] with *Daphnia* and *Hexagenia* to determine the toxicity of several contaminated sediments, and to confirm the suitability of the two

species as test organisms for screening sediments for acute toxicity. They found *Daphnia* to be more sensitive to sediment toxicants than *Hexagenia*, and in some paired sediment tests with and without *Hexagenia*, *Daphnia* died only in test chambers containing *Hexagenia*, probably because the higher turbidity created by the burrowing mayfly caused greater release of toxicants.

Malueg et al. [9] conducted tests using the Prater-Anderson apparatus with *Daphnia* using sediment from three areas contaminated with copper and other heavy metals. They found a good correlation between laboratory tests and field observations of the harmful impacts on the invertebrates, indicating that laboratory tests may predict freshwater sediment toxicity from samples collected from suspected areas. They also conducted tests using sediment from the Keweenaw Waterway, MI [10], and showed direct sediment-copper toxicity relationships with *Daphnia* and *Hexagenia*, and with distribution of benthic macroinvertebrates.

Cairns et al. (manuscript in preparation) conducted a series of tests using *Daphnia* with the same sediments used by Malueg et al. [9] but with two types of tests—the liquid phase elutriate test in aerated 250-ml beakers, and the solid phase sediment and water test in aerated 1,000-ml beakers. Both types of tests produced good data, with good control survival and replicates and with results similar to those of Malueg et al.

Cairns et al. [11] and Nebeker et al. (manuscript in preparation) conducted elutriate and solid phase beaker tests with *Daphnia*, *Chironomus*, *Hyalella*, *Gammarus* and *Hexagenia* using field-contaminated sediments and clean sediments artificially spiked with copper and cadmium. *Daphnia* was the most sensitive animal tested (48-h tests) while the other animals were generally similar in their sensitivity (10-d exposures), with no one animal being consistently more or less sensitive. Control survival was satisfactory for *Daphnia*, *Chironomus* and

*Hyalella* without addition of food. However, larger *Gammarus* and *Hexagenia* exhibited excessive control mortality in the beakers due to competition for space and cannibalism. Tests using the larger 4-liter jars, rather than the 1-liter beakers, were satisfactory for *Gammarus* and *Hexagenia*. Flow-through tests were also completed and compared to the static tests with cadmium-contaminated sediments (Nebeker et al., manuscript in preparation). Good survival of *Chironomus*, *Hyalella* and *Daphnia* occurred in flow-through tests, whereas significant mortality occurred in static tests, indicating that the animals were being killed by cadmium released into the water column and concentrated to higher levels in the static tests than in the flow-through tests.

A study by Schuytema et al. [12] with *Daphnia* using cadmium-spiked sediment slurries again demonstrated the usefulness of *Daphnia* as a test animal. Results of the research showed that, at least for cadmium and *D. magna*, free cadmium ion was causing most of the toxicity; cadmium bound to the particulates in suspension apparently was not available.

Tests conducted to determine the suitability of older *Daphnia* for sediment studies, because they are much easier to screen from turbid water and sediment (Nebeker et al., manuscript in preparation) showed that, in general, 5-d-old *Daphnia* had EC<sub>50</sub> values similar to the <24-h-old daphnids for copper, cadmium and cyanazine. The data suggest that the use of 5-d-old *Daphnia*, rather than <24-h-old animals, will not result in differences in sensitivity. A study by Nebeker et al. [2] conducted with *Chironomus* determined that 10-d-old second-instar larvae were most suitable for starting tests. They are large enough to work with for reliable results, are the most sensitive of the three larger larval instars, and allow two larval molts (second to third and third to fourth instar) during a 10-d acute exposure. However, they should be replaced by third-instar larvae in *in situ* tests, as third instar

larvae are larger, allowing use of larger-mesh screen for cages.

A study completed by Knight [13] sought to determine suitable freshwater aquatic invertebrates for use in sediment screening studies and to develop and validate methods using those animals selected. The freshwater clam *Corbicula fluminea* was found to be very useful for long-term field monitoring and bioaccumulation studies, and is now being employed in the Sacramento River delta area for that purpose. However, it was not found to be useful in acute studies because it often closes its shell when exposed to toxic materials. Several other candidate invertebrates were screened and the midge *Chironomus decorus* and the freshwater amphipod *Corophium stimpsoni* were chosen for further study. *Corophium* was found to do well in Sacramento River water but the addition of some chloride was needed in laboratory studies. Rearing, life cycle and toxicity studies with heavy metals were completed with the midge *Chironomus*, and it was recommended as a useful animal for screening potentially toxic sediments.

Tests were conducted to validate the usefulness of the *Chironomus*, *Daphnia* and *Hyalella* chronic tests, and the bioconcentration tests (Nebeker et al., manuscript in preparation). Three adult emergence tests with second-instar *Chironomus* were successfully completed, with 45 to 70% adult emergence from control and toxic sediments. One test starting with third-instar larvae averaged 65% emergence (range 56–74%); results were similar between 20-liter aquaria and 4-liter jars. Five *Daphnia* chronic tests (10-d) were completed successfully. Counting total number of adults and young after 10 d gave an average of 97% survival of adult *Daphnia* in the controls, and an average of 408 young were recovered from each test jar. Several bioconcentration tests and partial life cycle tests with *Hyalella* were completed, with recovery of adult animals ranging from 21 to 95%. However, it was difficult to recover young due to their small size, especially when sediment was not

screened prior to testing. *Chironomus* bioconcentration and larval survival tests were completed successfully, with larval recoveries ranging from 68 to 91%.

Nebeker and others at the Corvallis EPA laboratory are presently conducting tests with the annelid worm *Lumbriculus variegatus* to determine its usefulness as a sediment test organism for acute, chronic and bioconcentration tests. Studies to date show that the worms have a sensitivity similar to that of *Daphnia* in copper mine-effluent sediment, but are more tolerant of sediment from harbors containing mixed contaminants.

#### DISCUSSION AND LITERATURE REVIEW

Several excellent articles on culture, rearing and testing of the various test animals are available. *Standard Methods* [8] presents information on collecting and holding test animals and on testing procedures. The American Society for Testing and Materials [1,14] and Buikema et al. [15] give detailed procedures for culturing and rearing *D. magna*. Arthur [16] reviews bioassay procedures for amphipods with specific reference to *Gammarus*. Culture methods for *D. magna*, *H. azteca*, *G. lacustris*, *C. tentans* and *H. rigida* can be found in the *Manual for Culture of Selected Freshwater Invertebrates* [17]. Fremling and Mauck [18] also present methods for using nymphs of burrowing mayflies as toxicity test organisms.

Acute tests completed at this laboratory with freshwater invertebrates were conducted using some methods similar to those used successfully by others, such as the modified freshwater Prater-Anderson system [5,6], and the 1,000-ml beaker method used by Swartz et al. [19] for marine benthic invertebrates. The elutriate test using *D. magna* in aerated 250-ml beakers with water containing only dissolved materials is adapted from the procedures recommended by U.S. EPA-U.S. Army Corps of Engineers

[4], and has been validated by several researchers [20–23] as a successful tool for screening potentially toxic sediments.

A report by Plumb [23] on procedures for handling and chemical analysis of sediment and water samples provides state-of-the-art guidance on sampling, preservation and analysis of sediments. It is a summary of methods currently in use and should be consulted for detailed procedures for handling sediments up to when they are used in bioassays. The review does not give bioassay methods, but does briefly summarize the elutriate procedure and gives excellent references for all phases of sediment collection, handling and chemical analyses.

Chapman et al. [24] present a brief summary in a convenient tabular form of the current knowledge regarding the fate and effects of priority pollutants. They list persistence, accumulative capacity and volatility, and the environmental compartment (water, sediment or biota) in which pollutants will most likely be found. They emphasize that the overall task of developing both a comprehensive and cost-effective environmental monitoring program for priority pollutants can be greatly simplified and improved by first knowing the relative importance of each pollutant and the environmental compartment of major concern.

Adams et al. [25] used the midge *C. tentans* to define the key route of exposure (interstitial water, water column water, sediment or food) for kepone in partial life cycle static and flow-through tests, using survival, growth and bioaccumulation as endpoints. They used 3-liter aquaria containing 100 g dry sediment that had been screened through a No. 25 (710- $\mu$ m opening) standard sieve to remove large particles. They dosed the sediment with kepone using dry sediment to which acetone containing the desired amount of kepone was added. A slurry of 75 ml acetone (containing kepone) and 100 g soil was stirred and placed under a hood to evaporate the acetone. Water was then added to the sediment and 25 midge

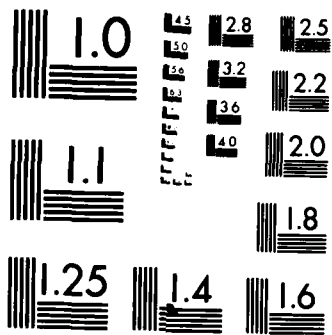
larvae were placed in the aquaria. They concluded that interstitial water was the primary route of exposure. Their results were excellent, and their sediment-spiking method using organics should work well as a standard method.

Seelye and Mac [26] present a literature review on the effects of dredging activities on freshwater organisms, and recommend the following: (a) Static tests should be avoided due to problems with low dissolved oxygen. (b) The elutriate test may not be of value. (c) Tests on dissolved or suspended particulate phases may not be of value. (d) Bioaccumulation studies should be conducted and the exposures should last at least 10 d, preferably > 30 d. (e) Whole sediment (solid phase) tests should be conducted and toxicity and bioaccumulation tests should be combined to reduce costs. (f) Sediment characteristics at the site should be defined because they greatly affect toxicant adsorption and availability. (g) Sublethal parameters such as reproductive success, growth abnormalities and avoidance should be considered for testing. (h) Use of flow-through tests should be encouraged. (i) Test animals should come in contact with the sediments. (j) Test 1 (elutriate) and test 2 (suspended particulates) of the U.S. EPA–U.S. Army Corps of Engineers methods manual [4] should not be used—only test 3, the whole sediment (solid phase) test, should be used.

We found that static tests were very useful and that aeration of the test containers eliminated the oxygen problem [11]. The elutriate test was a valuable addition to the solid phase test, because the combination showed when the sediment was toxic but was not releasing dissolved materials into the overlying water column: One of the toxic sediments from Wisconsin, with 27% organic content, was not toxic in the elutriate test but was toxic when animals had access to the solid phase sediment. Most of the sediments that caused mortality in test animals showed toxicity in both the elutriate and the solid phase tests, usually due to







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high dissolved concentrations that leached into the water (desorbed) from the sediment.

The question of freezing sediments versus storing them at 4°C until testing still needs resolution. A. Gahler (U.S. EPA, Region 10, Seattle, WA, personal communication) reported that freezing and defrosting alters release of nitrogen and phosphorus from sediments. J. Cummins (EPA Region 10, personal communication) observed that when frozen sediments are used toxicity is reduced by 20 to 30%. T. Dillon (poster presentation, SETAC Meeting, Arlington, VA, November 6-9, 1983) conducted studies on the effects of time and storage temperature (-22, 4 and 25°C) on sediment toxicity and recommended that sediment be stored at 4°C and used within 2 weeks if possible. We are presently conducting freezing (-20°C) versus 5°C storage tests to determine impacts on sediment chemistry and toxicity. Results to date show that freezing significantly reduces the toxicity to *Daphnia* of sediment spiked with copper. Caution is recommended in freezing sediment unless data are available to show what changes occur.

Bahnick et al. [20] conducted extensive acute 96-h tests and bioconcentration studies with *Hexagenia*, the amphipod *Pontoporeia* (native to the Great Lakes) and *Daphnia* using sediments from the Duluth-Superior Harbor and Lake Superior. Their procedures used sediment with overlying water (solid phase), tests with interstitial water and tests using elutriate water. The results demonstrated that the animals could be maintained successfully in complex test systems with good survival in controls, and in most of the sediments tested. *D. magna* was the most sensitive to the contaminated sediments.

Prater and Anderson [5,6] and Hoke and Prater [27] used a unique recirculating bioassay chamber and conducted an extensive series of bioassays to develop methodology and generate data. They used *Daphnia*, *Hexagenia* and other invertebrates, and screened many sediments from the Upper

Midwest and Great Lakes areas. Their methods were verified by Malueg et al. [7] as being useful in characterizing the toxicity of heavily contaminated sediments. Further attempts were made to sort out cause and effect relationships between chemical contaminants and biological responses [27,28] but without great success. They concluded that bulk chemistry correlated better with test species mortality than did elutriate (soluble) chemical measurements; however, that conclusion was disputed by Lee and Jones [29], who support the elutriate chemical test.

In laboratory tests, significant effects of contaminated sediment from Palestine Lake, IN, were demonstrated on the survival and growth of larvae of the midge *C. tentans* [30], and emergence of midge adults was reduced to one-third of control levels [31]. Shuba et al. [32] conducted several flow-through bioassays on kepone-contaminated sediments from Bailey Creek and the James River in Virginia, and also on sediments from a small stream receiving the Vicksburg, MS, sewage treatment plant effluent. Because their primary interest was in developing tests to predict effects of dredge-spoil disposal, they used a variety of sediment treatments, exposure times and test organisms (*Daphnia*, *Corbicula*, *Palaemonetes*, *Musculium* and *Lirceus*). They recommended *Palaemonetes*, *Daphnia* and *Musculium* as test species for acute toxicity studies and *Corbicula* for bioaccumulation studies. In our studies, we found *Daphnia* to be most sensitive, and Knight et al. [13] recommended *Corbicula* for bioaccumulation studies.

Marking et al. [33] conducted static toxicity tests with sediments from 10 sites on the upper Mississippi River, using *Gammarus*, *Procambarus*, *Chironomus*, *Physa*, *Truncilla* and *Sphaerium*. Sediments for 2 of the 10 sites were toxic to one or more test species, but no consistent responses of the test species were observed. Gannon and Beeton [34] conducted bioassays on sediments from nine Great Lakes harbors using the am-

phipod *Pontoporeia affinis*, an important component of the Great Lakes benthic community. About one-third of the sediments tested were toxic (50% mortality) to the test organism. Bailey and Liu [35] demonstrated the potential of the freshwater oligochaete *Lumbriculus variegatus* as a bioassay organism in water only and gave methods for rearing and testing. It has potential importance in toxicity and bioconcentration studies with contaminated sediments because it maintains intimate contact with the sediment.

Nearly all of the sediment toxicity investigations reported here used static exposure systems rather than flow-through tests, which seems appropriate as a conservative approach for predictive or assessment purposes. Evidence to date indicates that, generally, biologically available sediment contaminants are those released back into the water. A static exposure system allows the contaminants to accumulate and affect the test organism in a worst-case type of environment, and the potential toxicity of a sediment can be determined.

#### TEST SELECTION CRITERIA

Proper selection of test method or test animal is necessary to obtain the most useful information, especially with limited resources.

The choice of the bioconcentration test is obvious when there is a need to determine tissue levels of a chemical known to occur in the sediment under investigation. The decision as to which acute or chronic toxicity test to use may be more difficult.

The 48-h solid phase (sediment and water) beaker test with *Daphnia*, in conjunction with the elutriate test, is recommended as a relatively fast, simple and inexpensive approach to initial screening for acute toxicity. Elutriate and solid phase tests conducted together are valuable because the combination shows when the sediment is toxic but is not releasing dissolved

materials into the overlying water column. *Daphnia* generally have been shown to be the most sensitive animals used for sediment tests, especially to metals, and are the logical first choice. They are easy to rear and handle in the laboratory and there is a large database on *Daphnia* available from freshwater tests. The tests are relatively inexpensive, require little special equipment and are of short (48-h) duration. Even though they are planktonic, *Daphnia* do feed at the surface of the sediments and come in contact with particulate-bound toxicants.

*Hyalella* and *Chironomus* are the recommended benthic organisms for use in solid phase tests, as they are easy to rear and test in the laboratory. They maintain intimate contact with and burrow in the sediment, consistently exhibit high control survival and are very sensitive to toxic organic chemicals. If the sediment has a high organic content, a test with *Hyalella* or *Chironomus* in addition to one with *Daphnia* would be appropriate to determine toxicity of the solid phase sediment rather than soluble toxicants only. *Daphnia* and *Chironomus* can be tested together in the same beaker. *Gammarus* and *Hexagenia* are more useful in tests combining toxicity and bioconcentration, in larger containers, because of the greater biomass available for tissue analysis.

Because of greater organism diversity, use of all five test organisms at the same time (in separate containers, except for *Daphnia* and *Chironomus*) is a valuable way to characterize sediment toxicity. With two replicate containers per organism, only 16 beakers would be required, using one contaminated sediment sample and a control.

The chronic tests have a valuable function in determining if impairment of growth and reproduction may occur in sediments that do not exhibit acute toxicity, portending disruption of biological integrity and the subsequent loss of invertebrate populations from impacted freshwater areas.

## REFERENCES

1. American Society for Testing and Materials. 1982. Standard practice for conducting renewal life-cycle toxicity tests with *Daphnia magna* (Draft 1, Aug. Rita Comotto, E47.01).
2. Nebeker, A.V., M.A. Cairns and C.M. Wise. 1984. Relative sensitivity of *Chironomus tentans* life stages to copper. *Environ. Toxicol. Chem.* 3:151-158.
3. Nebeker, A.V., P. McKinney and M.A. Cairns. 1983. Acute and chronic effects of diflubenzuron (Dimilin) on freshwater fish and invertebrates. *Environ. Toxicol. Chem.* 2:329-336.
4. U.S. Environmental Protection Agency/U.S. Army Corps of Engineers. 1977. Ecological evaluation of proposed discharge of dredged material into ocean waters. Environmental Effects Laboratory, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
5. Prater, B.L. and M.A. Anderson. 1977. A 96-hour bioassay of Otter Creek, Ohio. *J. Water Pollut. Control Fed.* 49:2099-2106.
6. Prater, B.L. and M.A. Anderson. 1977. A 96-hour bioassay of Duluth and Superior harbor basins (Minnesota) using *Hexagenia limbata*, *Asellus communis*, *Daphnia magna*, and *Pimephales promelas* as test organisms. *Bull. Environ. Contam. Toxicol.* 18:159-168.
7. Malueg, K.W., G.S. Schuytema, J.H. Gakstatter and D.F. Krawczyk. 1983. Effect of *Hexagenia* on *Daphnia* response in sediment toxicity tests. *Environ. Toxicol. Chem.* 2:73-82.
8. American Public Health Association/American Water Works Association/Water Pollution Control Federation. 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. APHA, Washington, DC, pp 615-714.
9. Malueg, K.W., G.S. Schuytema, J.H. Gakstatter and D.F. Krawczyk. 1984. Toxicity of sediments from three metal-contaminated areas. *Environ. Toxicol. Chem.* 3:279-291.
10. Malueg, K.W., G.S. Schuytema, D.F. Krawczyk and J.H. Gakstatter. 1984. Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw Waterway, Michigan. *Environ. Toxicol. Chem.* 3:233-242.
11. Cairns, M.A., A.V. Nebeker, J.H. Gakstatter and W. Griffiths. 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. *Environ. Toxicol. Chem.* 3:435-446.
12. Schuytema, G.S., P.O. Nelson, K.W. Malueg, A.V. Nebeker, D.F. Krawczyk, A.K. Ratcliff and J.H. Gakstatter. 1984. Toxicity of cadmium in water and sediment slurries to *Daphnia magna*. *Environ. Toxicol. Chem.* 3:293-308.
13. Knight, A.W. 1984. The evaluation of contaminated sediments employing selected benthic freshwater invertebrates. Final Report of the U.S. EPA Cooperative Agreement No. CR-808424, University of California, Davis, CA. Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.
14. American Society for Testing and Materials. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E 729.80. American Society for Testing and Materials, Philadelphia, PA.
15. Buikema, A.L., Jr., J.G. Geiger and D.R. Lee. 1980. *Daphnia* toxicity tests. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. American Society for Testing and Materials, Philadelphia, PA, pp. 48-69.
16. Arthur, J.W. 1980. Review of freshwater bioassay procedures for selected amphipods. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. ASTM, Philadelphia, PA, pp. 98-108.
17. Lawrence, S.G., ed. 1981. *Manual for the Culture of Selected Freshwater Invertebrates*. Can. Spec. Publ. Fish. Aquat. Sci. 54:169 p.
18. Fremling, C.R. and W.L. Mauck. 1980. Methods for using nymphs of burrowing mayflies (Ephemeroptera, *Hexagenia*) as toxicity test organisms. In A.L. Buikema, Jr., and John Cairns, Jr., eds., *Aquatic Invertebrate Bioassays*. ASTM STP 715. ASTM, Philadelphia, PA, pp. 81-97.
19. Swartz, R.C., W.A. DeBen, K.A. Sercu and J.O. Lamberson. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Pollut. Bull.* 13:359-364.
20. Bahnick, D.A., W.A. Swenson, T.P. Markee, D.J. Call, C.A. Anderson and R.T. Morris. 1980. Development of bioassay procedures for defining pollution of harbor sediments. Final Report of the U.S. EPA Project No. R804918-01. Center for Lake Superior Environmental Studies, University of Wisconsin, Superior, WI. Also: Project Summary. EPA-600/S3-81-025. June 1981. Environmental Research Laboratory, Duluth, MN.
21. Lee, G.F., R.A. Jones, F.Y. Saleh, G.M. Mariani, D.H. Homer, J.S. Butler and P. Bandyopadhyay. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Volume II: Data report. Technical Report D78-45. U.S. Army Corps of Engineers, WES, Vicksburg, MS.
22. Jones, R.A. and G.F. Lee. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Volume I: Discussion. Technical Report D78-45. U.S. Army Corps of Engineers, WES, Vicksburg, MS.
23. Plumb, R.H., Jr. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
24. Chapman, P.M., G.P. Romberg and G.A. Vigers. 1982. Design of monitoring studies for priority pollutants. *J. Water Pollut. Control Fed.* 54:292-297.
25. Adams, W.J., R.A. Kimerle and R.G. Mosher. 1983. Aquatic safety assessment of chemicals

- sorbed to sediments. Presented at American Society for Testing and Materials 7th Annual Aquatic Toxicology Symposium, April 17-19, 1983, Milwaukee, WI.
26. Seelye, J.G. and M.J. Mac. 1982. Bioaccumulation of toxic substances during dredging. Draft Final Report of the Interagency Agreement AD-14-F-1-529-0. U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, IL.
  27. Hoke, R.A. and B.L. Prater. 1980. 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.
  28. Laskowski-Hoke, R.A. and B.L. Prater. 1981. Dredged material evaluation: Correlations between chemical and biological procedures. *J. Water Pollut. Control Fed.* 53:1260-1262.
  29. Lee, G.F. and R.A. Jones. 1982. Discussion of dredged material evaluations: Correlations between chemical and biological procedures. *J. Water Pollut. Control Fed.* 54:406-407.
  30. Wentzel, R.A., A. McIntosh and G. Atchison. 1977. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). *Hydrobiologia* 56:153-156.
  31. Wentzel, R.A., A. McIntosh and P.C. McCafferty. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediments. *Hydrobiologia* 57:195-196.
  32. Shuba, P.J., H.E. Tatum and J.E. Carroll. 1978. Biological assessment methods to predict the impacts of open-water disposal of dredged material. DMRP Technical Report No. D-78-50, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.
  33. Marking, L.L., V.K. Dawson, J.L. Allen, T.D. Bills and J.J. Rach. 1981. Biological activity and chemical characteristics of dredge material from ten sites on the upper Mississippi River. Summary report. U.S. Fish and Wildlife Service, LaCrosse, WI.
  34. Gannon, J.E. and A.M. Beeton. 1971. Procedures for determining the effects of dredged sediments on biota-benthos viability and sediment selectivity tests. *J. Water Pollut. Control Fed.* 43:392-398.
  35. Bailey, H.C. and D.H.W. Liu. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. J.C. Eaton, P.R. Parrish, and A.C. Hendricks, eds., In *Aquatic Toxicology*. ASTM STP 707. American Society for Testing and Materials, Philadelphia, PA, pp. 205-215.

## METHODOLOGY FOR ASSESSMENT OF POTENTIAL MUTAGENICITY OF DREDGED MATERIALS

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### ABSTRACT

Dredged material disposal criteria are currently based on measurement of chemical constituents, toxicity and biostimulation, but not on the presence of mutagenic materials. Extracts of sediments collected from Southwestern Lake Michigan ranged from non-mutagenic to highly mutagenic when assayed by the Ames Test. It is suggested that this test be used to provide additional information of value for the issuance of permits for dredging activities.

### INTRODUCTION

Approximately  $3 \times 10^8 \text{ m}^3$  of sediment are dredged annually from United States waterways to maintain desired navigation depth. As dredged materials may be from areas polluted with such substances as biocides, heavy metals and toxic organic chemicals, the environmental effects due to chemical contaminants associated with the disposal of this material is of major importance (1). Under Section 404 of the Federal Water Pollution Control Act (P.L. 92-500) the Secretary of the Army, acting through the Chief of the Corps of Engineers, is authorized to issue permits for the disposal of dredged material into navigable waters at specified disposal sites. As part of the permit application procedure an ecological evaluation must be performed using Environmental Protection Agency guidelines for evaluating the potential environmental impact of dredging disposal activity (2). The evaluation considers environmental impact assessments and applicable coastal zone management programs and river basin plans. To determine the degree of contamination of the sediment, chemical analysis of the sediment and bioassays for toxicity, stimulation, inhibition and bioaccumulation effects may be required. No bioassay for mutagenic potential of dredged material is currently in use in the evaluation procedure. The Ames Test or Salmonella/Mammalian Microsome Mutagenicity Test has been suggested for general use in screening environmental samples to determine their mutagenic potential and has been widely used for the analysis of drinking waters (3-9).

This study was undertaken to provide data on the presence of mutagenic substances in sediments from the southwestern shoreline areas of Lake Michigan and to aid in determining whether or not this type of information could prove of value in assessing the environmental impact of dredged material. Surficial aquatic sediments, from both harbor and recreational beach areas were collected, extracted and analyzed for mutagenicity using the Ames test.

## MATERIALS AND METHODS

### Sample Collection

A series of ten sediment samples were collected on December 14, 1978 from the deck of the Argonne National Laboratory's research vessel "Ecos". The sampling locations are shown in Figure 1. A ponar dredge was used for the sampling. The top 2-3 inches of each sediment were removed, placed in a plastic container, transported to the Illinois Institute of Technology and stored at 2-4°C.

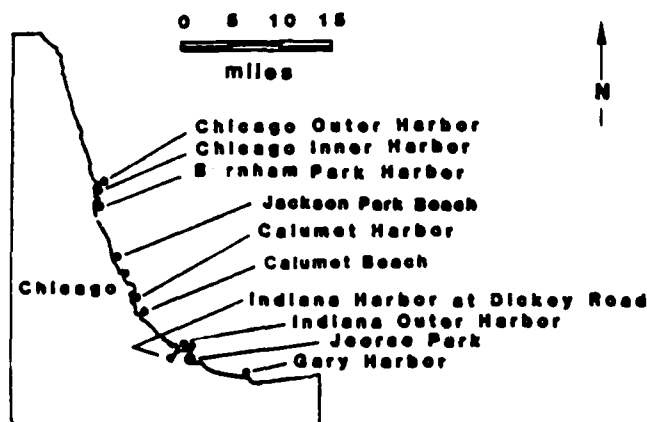


Figure 1. Sampling Locations

### Sample Preparation and Extraction

After thorough mixing of the wet sediment one portion was transferred to a preweighed extraction thimble and a second portion was transferred to a preweighed crucible. The crucible and wet sediment were dried at 103°C to permit the dry weight of sediment to be determined. The ratio of dry to wet weight sediment in the crucible was used to convert the wet weight of sediment in the extraction thimble to an equivalent dry weight of material.

The thimble containing the wet sediment was placed in a soxhlet extraction apparatus and extracted with 100 ml methanol (10). After 24 hours of extraction, 30 ml benzene was added to the extraction flask and the extraction was continued for an additional 24 hours. The extract was transferred to a beaker and the solvent evaporated by passing a stream of air over the liquid surface. The residue was taken up in 30 ml of dimethylsulfoxide (DMSO) and this residue solution was stored at 2-4°C until used.

### Mutagenicity Testing

Preliminary testing with Salmonella typhimurium strains TA 98, TA 100 and TA 1538 indicated that the greatest reversion rate for mutagens extracted from sediment and the lowest reversion rate for negative controls was obtained for strain TA 1538. Strain TA 1538, which was used for all further work, requires histidine for growth unless it is reverted to a histidine synthesizing form by a wide variety of mutagens (9). The bacterial tester strain which was obtained from Dr. Barry Commoner, Washington University, St. Louis, Missouri was tested for its genetic mutations of histidine requirement, rfa character and uvrB deletion using the procedures of Ames et al.



Samples were activated by Arochlor 1254 induced rat liver S-9 mixture purchased from Litton Bionetics, Kensington, Maryland. The S-9 mixture was prepared by the procedure of Ames *et al.* (11).

The plate incorporation technique (11) was used for all mutagenesis assays. To 2 ml of molten top agar at 45°C were added 0.1 ml of an overnight nutrient broth culture of TA 1538, 0.25 ml of the sample to be tested, and 0.5 ml of the S-9 mix. The contents were mixed and spread uniformly on a minimal Vogel-Bonner agar plate. The plates were placed in a dark 37°C incubator for 2 days after which the bacterial colonies were counted.

For each sediment extract a series of five samples were prepared in duplicate so that the presence or absence of a dose-response effect could be established. Four sample concentrations were prepared by diluting 0.01, 0.1, 0.2 and 0.5 ml of the residue extract solution to 1.0 ml with DMSO. The fifth sample concentration was the undiluted residue extract solution.

Ames tests were also conducted for controls in which the sample was pure DMSO and for a series of six concentrations of the known mutagen, 2-acetylaminofluorene, for which the maximum dose was 50 µg per assay. Finally, the mutagenic activity of the undiluted residue extract of the samples was tested without activation by the S-9 mix.

## RESULTS AND DISCUSSION

### Ames Test Results

Results of the Ames tests for the ten samples are summarized in Table 1. No significant response greater than that for the controls was found for samples which were not activated by S-9. Linear dose-response relationships for samples assayed with S-9 mix activation were observed (Figure 2) over the range of dosages tested for only four of the sediments: Jackson Park Beach, Calumet Harbor, Indiana Outer Harbor and Gary Harbor. The linearity of the response was established by determining a least-squares linear regression and testing to determine if the slope of the regression was statistically different from zero. In each of the four cases the slope was significantly different at the 95% confidence level.

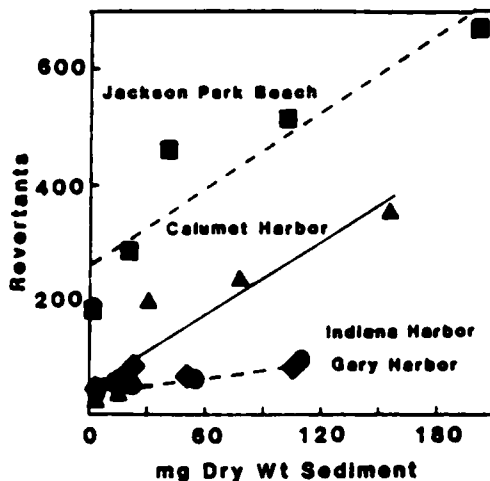


Figure 2. Samples with linear dose-response relationships.

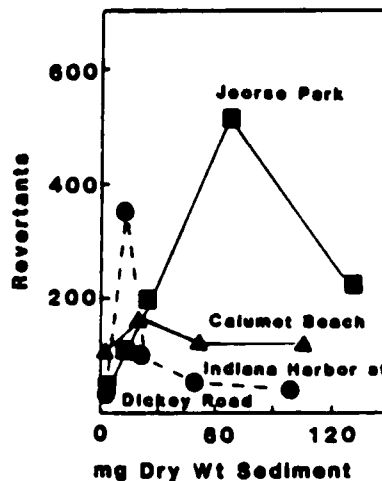


Figure 3. Samples with maxima in dose-response relationships.

TABLE 1. RESULTS OF MUTAGENICITY TESTING OF LAKE MICHIGAN SEDIMENTS.

Sample Number	Location	Wet Weight Sediment Extracted (g)	Maximum Number of Revertants $R_{max}$	$\frac{R_{max}}{C}$ +
1	Chicago Inner Harbor	41.7	28	1.0
2	Chicago Outer Harbor	37.3	26	1.0
3	Burnham Park Harbor	36.4	30	1.1
4	Jackson Park Beach	43.0	675	25.0
5	Calumet Harbor	44.7	351	13.0
6	Calumet Beach	31.3	159	5.9
7	Indiana Outer Harbor	35.1	89	3.3
8	Indiana Harbor at Dickey Road	34.2	351	13.0
9	Jeorse Park	34.7	509	18.9
10	Gary Harbor	34.6	89	3.3

\* Average of two samples

+ Ratio of maximum number of revertants to number of revertants for DMSO control

Two of the samples, Indiana Harbor at Dickey Road and Jeorse Park, first show an increase followed by a decline in the number of revertants as the quantity of material per plate was increased (Figure 3). The sample collected from Calumet Beach shows a similar, but less clear, result (Figure 3). The decreases may be due to the presence of toxic or inhibitory substances in the samples. Ames *et al.* (11) have observed similar types of curves with certain carcinogens. They attributed the observed response to the inactivation by the mutagen of many essential genes on the chromosomes.

For three samples, Chicago Inner Harbor, Chicago Outer Harbor and Burnham Park Harbor, based on the value of the correlation coefficient there was not a statistically significant increase in the number of revertants with respect to the dosage.

A dose-related response has been used by some investigators (9-13) to indicate positive mutagenicity results. As recommended by Lampietti and Marcus (13) we have considered the ratio of the total number of revertants to the number of revertants for the control to provide a more quantitative basis to evaluate the degree of mutagenicity. Values of this R/C ratio computed for the dosage having the greatest average reversion rate are tabulated in Table 1.

Comanor (14) has found for a series of compounds that a R/C ratio greater than 3.5 indicates, with a probability of 95%, that the substance is a carcinogen or presumptive carcinogen and a R/C ratio of 3.0 or less indicates, with a reliability of 83%, that the substance is non-carcinogenic. The three samples for which there was not a dose related response had R/C ratios less than 3.0 which indicates that carcinogenic substances are probably absent. The Indiana Outer Harbor and the Gary Harbor samples had R/C ratios of 3.3, a value too low to conclude that there are substances present which, with a 95% probability, are carcinogenic. The remaining samples had R/C ratios ranging from 5.9 to 25.0, suggesting that carcinogenic substances may be present in these samples.

#### Evaluation of Ames Test Results

The maximum observed autagenic response provides a semi-quantitative indication of sample mutagenicity but does not allow for the comparison of different samples

from a common reference point. Furthermore, for some samples there is no maximum in the dose-response curve. For these samples the maximum mutagenicity is a maximum imposed by the conditions selected for the test. Furthermore, it is not always practical to extract the same equivalent amount of dry sediment and, as seen in Table 2, the equivalent dry weight of sediment extracted for these ten sediments varied by more than a factor of 2. A quantitative expression of mutagenic potential is important for making decisions regarding risk assessment and in priority ranking areas of concern.

TABLE 2. REVERSION RATE PER MG SEDIMENT AND EQUIVALENT REVERSION RATE

Sample Number	Location	Dry Weight Sediment Extracted (g)	$\frac{R_{max}}{\text{mg Dry Wt Sediment}}$	Equivalent $\frac{\mu\text{g AAF}}{\text{mg Dry Wt Sediment}}$
1	Chicago Inner Harbor	22.2	0.03	0.002
2	Chicago Outer Harbor	16.0	-0.01	-0.001
3	Burnham Park Harbor	12.7	0.06	0.004
4	Jackson Park Beach	24.2	3.21	0.218
5	Calumet Harbor	18.6	2.09	0.142
6	Calumet Beach	12.4	6.38	0.434
7	Indiana Outer Harbor	12.7	0.59	0.040
8	Indiana Harbor at Dickey Road	11.6	33.40	2.272
9	Jeorse Park	15.6	7.39	0.503
10	Gary Harbor	12.1	0.61	0.041

\* Obtained by dividing the value of  $\frac{(R_{max} - C)}{\text{mg Dry Weight Sediment}}$  by the slope of the AAF dose-response curve (14.7 revertants/ $\mu\text{g AAF}$ )

To compare the relative mutagenic responses of the series of samples, it is necessary to compare the net reversion rate per unit mass dry weight of sediment extracted. These data are also presented in Table 2. A similar trend can be seen for this treatment as was seen for the R/C ratio. The Chicago Harbor samples and Burnham Harbor sample which did not have significant dose-related responses and which had R/C ratios nearly unity had less than 0.1 net revertant per mg dry weight of sediment. The remaining five samples had R/C ratios exceeding 3.5 and more than 1.0 net revertants per mg dry weight of sediment.

Consideration of the net reversion rate per mg dry weight of sediment results in a different ranking of samples than that provided by the R/C ratio. The sample collected from Jackson Park Beach had the largest R/C ratio but only the fourth greatest value of net revertants per mg dry weight of sediment. The sample collected from Indiana Harbor at Dickey Road had the highest number of revertants per mg dry weight of sediment but it had only the third highest R/C ratio.

Although determining the net revertants per mg dry weight of sediments provides a basis for the comparison of different sediments it does not allow analyses conducted at different times or those for which different S-9 mixtures were used to be compared. Analysis of a compound which is mutagenic only after activation provides a means to verify the activity of the S-9 mix and to correlate the response obtained for a unit weight of sediment to that of the standard compound.

We selected 2-acetylaminofluorene (AAF) for this purpose. A linear dose-response curve verified the activity of the S-9 mix. Using 50  $\mu\text{g AAF}$  and 50  $\mu\text{l S-9}$  per plate, there were 741 net revertants. For the same quantities of AAF and S-9 Ames has reported 9,600 (11) and 13,000 (12) revertants. He has also reported 8,600

revertants with 100 µg AAF and 50 µl of S-9 (15). The mutagenesis titrations performed for strain TA-98 by Litton Bionetics on the lot of S-9 used during this work showed 600 revertants per plate for 20 µg AAF and 50 µl of S-9. In addition to containing the same mutations as TA 1538, strain TA 98 contains the pKM 101 plasmid making it more sensitive to some mutagens (11). Our value is consistent with that for the titration by Litton Bionetics. The difference between the number of revertants found for AAF in this study and those reported by Ames is likely due to a difference in S-9 activity. As previously noted, because of such differences it is desirable to relate experimental results for environmental mixtures to an equivalent amount of a single mutagenic compound. Because a number of enzymes in S-9 may be involved in the activation of the mutagens in a complex sample, all mutagens may not be activated to the same extent by a given batch of S-9.

We determined the slope of the dose-response curve for the AAF to be 14.7 revertants per µg AAF. Using this factor the responses for the sediment samples were converted to equivalent amounts of AAF. These results are presented in Table 2. These data allow comparison of the sediments collected during this study with sediments collected in the future and analyzed with a different S-9 mixture.

#### SUMMARY AND CONCLUSIONS

The results of this study show that sediments in several areas along the southwestern shore of Lake Michigan contain significant levels of mutagenic substances as determined in the Ames Test. By interpreting the results on the basis of the number of revertants per mass dry weight of sediment and then converting that response to an equivalent amount of the known carcinogen, 2-acetylaminofluorene (AAF), results of sediment bioassays can be compared on a quantitative basis.

As an inexpensive and rapid screening technique, this test should be an important addition to the chemical tests and bioassays currently performed. Mutagenicity testing results will be of benefit to those making decisions regarding the need for additional chemical testing which is both time-consuming and costly. Most important, such results will aid in the evaluation process in the issuance of permits for dredging activities.

#### REFERENCES

1. G. F. Lee, *Environ. Sci. Technol.*, 10, 334-338 (1976).
2. D. D. Smith, *Environ. Sci. Technol.*, 10, 328-333 (1976).
3. B. J. Dutka and K. Switzer-House, *J. Great Lakes Res.*, 4, 237-241 (1978).
4. E. P. Flanagan and H. E. Allen, *Bull. Environ. Contam. Toxicol.*, 27, 765-772 (1981).
5. S. M. Grimm-Kibalo, B. A. Glatz and J. S. Fritz, *Bull. Environ. Contam. Toxicol.*, 26, 188-195 (1981).
6. H. J. Kool, C. F. van Kreijl, H. J. van Kranen and E. de Greef, *Chemosphere*, 10, 85-98 (1981).
7. H. J. Kool, C. F. van Kreijl and H. J. van Kranen, *Chemosphere*, 10, 99-108 (1981).
8. J. C. Loper, *Mutation Res.*, 76, 241-268 (1980).
9. W. Pelon, B. F. Whitman and T. W. Beasley, *Environ. Sci. Technol.*, 11, 619-623 (1977).
10. W. Giger and M. Blumer, *Anal. Chem.*, 46, 1663-1671 (1974).
11. B. N. Ames, J. McCann and E. Yamasaki, *Mutation Res.*, 31, 347-364 (1975).
12. B. N. Ames, W. E. Durston, E. Yamasaki and F. D. Lee, *Proc. Nat. Acad. Sci. (U.S.A.)*, 70, 2281-2285 (1973).
13. F. J. de Serres and M. D. Shelby, *Science*, 203, 563-565 (1979).
14. B. Commoner, *Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Noncarcinogenic Chemicals*, EPA-600/1-76-022 (1976).
15. W. E. Durston and B. N. Ames, *Proc. Nat. Acad. Sci. (U.S.A.)*, 71, 737-741 (1974).

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DEPARTMENT OF THE ARMY  
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March 14, 1985

REPLY TO  
ATTENTION OF.

SUBJECT: Workshop to Evaluate Sediment Bioassessment Techniques

Mr. Tom Dillon  
Environmental Laboratory  
Waterways Experiment Station, Corps of Engineers  
P.O. Box 631  
Vicksburg, Mississippi 39180

Dear Tom:

1. As we discussed on the phone a few days ago, I will be standing in for Jim Mansky at the upcoming workshop to be held 16-18 April 1985 at Milwaukee, Wisconsin. As you requested in your letter of 14 January 1985, I have included descriptions of appropriate bioassessment techniques used in the New York District relating to open water (ocean) disposal of dredged material.

2. Since 1976, the New York District (NAN) has been actively involved with bioassessment techniques to determine proposed dredged material suitability for open ocean disposal. NAN has adhered to the Corps Implementation Manual for Section 103 of the Ocean Dumping Act. As is delineated in the Manual, NAN has modified these tests to address the harbor's specific conditions and concerns, among which are ocean disposal as a controversial option and the lack of feasible alternatives.

a. NAN has used the three phase bioassay to assess the potential toxicity of dredged material. When mobilized, either during dredging or disposal, dredged material may transfer contaminant into the water (1) in a dissolved state (liquid phase bioassay), (2) by being adsorbed onto suspended particles (suspended particulate phase) or (3) within interstitial water in material deposited on the bottom (solid phase). Three different species, which represent typical organisms found in the harbor, are tested for each phase. Their feeding or life habits dictate their use in one or another of the phases.

b. These bioassays measure mortality of the tested material relative to a control. The two are compared and an LC 50 calculated. This number, which is calculated for each test species, is a measure of toxicity.

c. The calculated concentrations of the tested material which would have an unacceptable toxicity on these representative organisms are compared with the theoretically calculated concentrations of the material which would be expected during dredging or disposal. The determination is then made whether and to what degree the dredging or disposal

techniques have to be modified to reduce their potential toxicity. The determination may also be made, as can be the case with the solid phase bioassay, that the material can not be open water disposed.

d. Test, control, and reference comparisons are made for the solid phase bioassay. The reference differs from the clean control, which is designed to determine the validity of the test. Instead the reference is material which is subject to harborwide environmental degradation, but is free of the influence of dredged material disposal. Comparison of the reference and test material assesses whether dredged material disposal per se has the potential for further degrading the disposal site.

e. Bioaccumulation analyses are conducted on surviving solid phase bioassay test organisms. The test organism tissues are analysed for selected contaminants of concern to determine the likelihood that these contaminants may be passed along the aquatic food web and concentrated at higher trophic levels.

f. The bioaccumulation test yields body burden concentrations as parts per million of wet tissue. A special literature study was done to interpret these tissue levels. Criteria for different test organisms were established for each contaminant of concern. These were determined by averaging the tissue concentrations found in field collected organisms in New York Harbor which is subject to a variety of adverse environmental influences exclusive of dredged material disposal. The resultant criteria, as indicated in the Interpretive Guidance, serve to determine appropriate management strategies for open water disposal.

3. If you wish any further elaboration of the above, please contact me at FTS 264-5622. I look forward to seeing you at the workshop.

Sincerely,



Rich Krauser



**US Army Corps  
of Engineers**  
New York District



**GUIDANCE FOR PERFORMING TESTS  
ON DREDGED MATERIAL TO BE DISPOSED OF  
IN OCEAN WATERS**

**PREPARED BY :**

U. S. Army Corps of Engineers  
Regulatory Branch  
Water Quality Compliance Section  
26 Federal Plaza  
New York, NY 10278-0090

**IN CONJUNCTION WITH :**

EPA, Region II  
26 FEDERAL PLAZA  
NEW YORK, NY 10278-0012

**EFFECTIVE AS OF :**

21 December 1984





**US Army Corps  
of Engineers**

New York District  
26 Federal Plaza  
New York, N.Y. 10278  
ATTN: REGULATORY BRANCH

# Public Notice

In replying refer to:

Public Notice No. 11897-RQ

Published: Nov 20, 1981 Expires:

To Whom It May Concern:

The New York District of the Corps of Engineers (COE/NYD) and the U. S. Environmental Protection Agency (EPA), Region II, announce a new edition of the manual entitled Guidance for Performing Tests on Dredged Material to be Disposed of in Ocean Waters (Guidance Manual). This manual supersedes the edition dated 18 April 1982 with the same title. It will be revised periodically in order to incorporate modifications to the testing requirements.

The enclosed manual presents sediment testing guidelines for applicants who wish to dredge and dispose of dredged material in the Atlantic Ocean or in Long Island Sound. Department of the Army approval is required to dispose of dredged material into ocean waters; this manual includes administrative requirements for processing that application (see page iii). It also informs applicants of specific procedural items, such as bioassay organisms, chemical constituents required for analysis in bioaccumulation, etc.

Since this Guidance Manual adapts testing procedures to regional situations, it should be used in conjunction with the national Implementation Manual entitled Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters. The Implementation Manual was developed jointly by the Corps of Engineers and EPA; it defines procedures for evaluating potential environmental impacts associated with ocean disposal of dredged material.

This revised Manual, entitled Guidance for Performing Tests on Dredged Material to be Disposed of in Ocean Waters, will become effective December 21, 1984.

Questions and suggestions regarding any aspects of the Guidance Manual should be directed to:

U. S. Army Corps of Engineers  
New York District  
Regulatory Branch  
Water Quality Compliance Section  
26 FEDERAL PLAZA  
NEW YORK, NEW YORK 10278-0090

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## MANUAL

### INTRODUCTION

The enclosed material presents the sediment testing guidelines for permit applicants who wish to dispose of dredged material in the Atlantic Ocean OR LONG ISLAND SOUND. It also includes other administrative requirements for processing an application for Department of the Army approval. These guidelines have been prepared by the New York District Corps of Engineers (COE/NYD) in cooperation with Region II of the Environmental Protection Agency (EPA). THIS MANUAL WILL BE REVISED PERIODICALLY TO INCORPORATE MODIFICATIONS OF THE TESTING REQUIREMENTS. CHANGES MADE IN THIS GUIDANCE MANUAL SINCE THE 18 APRIL 1982 REVISIONS ARE TYPED IN "CAPITAL LETTERS".

In accordance with Section 227.27(b) of EPA's Ocean Dumping Regulations and Criteria (Federal Register, Vol. 42, No. 7, Tuesday, 11 January 1977) an Implementation Manual entitled Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters was developed jointly by the COE and EPA to define procedures for evaluating potential environmental impacts associated with ocean disposal of dredged material. The Implementation Manual presents national guidance concerning technical procedures and "is intended to encourage continuity and cooperation between COE Districts and EPA Regions in evaluative programs for Section 103 permit activities." Though the Implementation Manual presents detailed procedures for conducting tests required by EPA's Ocean Dumping Criteria, additional guidance is necessary to adapt the procedures to regional situations. For instance, regional guidance is needed to inform applicants of specific procedural items such as selection of bioassay organisms, chemical constituents required to be analyzed in bioaccumulation tests, etc. In addition, the manual summarizes the tests to be performed and the types of data to be submitted to the COE/NYD so as to avoid any unnecessary confusion and possible delays in the permit review process through the submission of improper data.

This manual does not attempt to modify any procedural aspect of the Implementation Manual. Questions regarding any aspect of the testing requirements should be directed to:

U. S. Army Corps of Engineers  
New York District  
Regulatory Branch  
Water Quality Compliance Section  
26 Federal Plaza  
New York, NY 10278-0090

ADMINISTRATIVE REQUIREMENTS

When Applying for Department of the Army Approval  
To Dispose of Dredged Material Into Ocean Waters

1. FIRST, THE APPLICANT MUST SUBMIT A REQUEST FOR AUTHORIZATION TO DISPOSE OF DREDGED MATERIALS AT EITHER THE OCEAN DISPOSAL SITE OR THE LONG ISLAND SOUND SITE, WHICHEVER IS APPROPRIATE. AT THAT TIME, THE FOLLOWING INFORMATION MUST BE INCLUDED:

- a. Current information regarding the need for dredging, including volume and area to be dredged, extent of shoaling, interruption or changes in standard operations resulting from shoaling, any available documentation showing problems resulting from the shoaling, and any other pertinent information.
- b. The applicant must study alternate methods and means of disposing of the dredged material, and must include current documentation of this study and justification of the environmental and/or economic reasons for having rejected these alternatives.
- c. If the request is being made under an existing Department of the Army maintenance dredging permit, include the permit number and a short description of the last maintenance dredging performed.
- d. Dimensions of the dump vessel (length, width and volume of hopper) AND THE type of dump vessel (split hull or pocket) the applicant plans to use.
- e. Two copies of an 8 $\frac{1}{2}$ " x 11" map showing the area to be dredged, the specific location of the proposed sediment sampling sites, AND A DETAILED BATHYMETRIC DESCRIPTION OF THE SOUNDINGS.

2. UPON SUBMITTING THIS INFORMATION, THE APPLICANT MUST ARRANGE A MEETING WITH COE/NYD PERSONNEL TO DISCUSS THE ADEQUACY OF THE PROPOSED SAMPLING DESIGN. COE/NYD RESERVES THE RIGHT TO MODIFY THE SAMPLING DESIGN, AS WELL AS THE SERIES OF TESTS REQUIRED.

3. PRIOR TO COMMENCEMENT OF SAMPLING, THE APPLICANT MUST SUBMIT TO THE COE/NYD THE NAMES OF THE ANALYTICAL CONTRACTORS AND SUBCONTRACTORS WHO WILL BE CONDUCTING THE BIOLOGICAL AND-CHEMICAL ANALYSES.

4. WHEN SEDIMENT TESTING HAS BEEN COMPLETED, THE APPLICANT MUST SUBMIT TWO COPIES OF THE TESTING REPORT TO COE/NYD. THIS REPORT MUST INCLUDE RAW DATA FOR ALL TESTS (GRAIN SIZE ANALYSES, BIOASSAYS, BIOACCUMULATION ANALYSES, CHEMICAL ANALYSES OF SITE WATER AND ELUTRIATE, AND BULK SEDIMENT ANALYSES), AN 8 $\frac{1}{2}$ " x 11" MAP OF THE AREA TO BE DREDGED SHOWING THE SPECIFIC LOCATIONS OF SEDIMENT AND WATER SAMPLING SITES, AND THE NAME OF THE LABORATORY(S) WHICH PERFORMED THE TESTS. ALL TESTING AND QUALITY CONTROL PROCEDURES MUST BE DESCRIBED, AND ANALYTICAL METHODS MUST BE SPECIFIED.

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5. FOR MORE DETAILS, CONSULT PAMPHLET EP1145-2-1 (NOV 1977), USACOE PERMIT PROGRAM, A GUIDE FOR APPLICANTS. THIS PAMPHLET IS AVAILABLE AT THE FOLLOWING ADDRESS:

U.S. ARMY CORPS OF ENGINEERS  
REGULATORY BRANCH  
26 FEDERAL PLAZA  
NEW YORK, NEW YORK 10278-0090

## MANUAL

### A. Selection of Sampling Sites

Selecting the proper number and location of sampling sites within the area to be dredged is a crucial step in the testing procedures. As a GENERAL RULE, a minimum of 3 sampling sites MUST be used. IN ADDITION, the following factors must be considered when choosing a sampling scheme.

1. The heterogeneity of the material to be dredged must be considered. If the material varies on the horizontal and/or vertical plane, more sampling sites are required so that the composited material reflects these differences. If the material varies greatly with depth, or if "new work" dredging is being undertaken, the applicant MUST include additional core samples of the composited material in order to REFLECT these differences.

2. The applicant must consider the existence of point source discharges in the area to be dredged, or other causes for concern such as historical occurrence of spills of oil or toxic or bioaccumulative chemicals, and outfalls which may affect the area to be dredged including sewage, storm water, industrial, municipal, commercial or residential discharges into the waterway. The intent of the Ocean Dumping Criteria is to identify and limit the ocean disposal of dredged material which is hazardous to the marine environment. The applicant acting in "good faith" is under AN OBLIGATION to develop a sampling scheme which adequately reflects those ends. Notwithstanding these "good faith" efforts, THE EPC/NYD REQUIRES REVIEW OF THE SAMPLING SCHEME PRIOR TO IMPLEMENTATION BY COE/NYC PERSONNEL FOR ADEQUACY to insure that these considerations have been fulfilled.

3. The applicant MUST supply an 8-1/2" x 11" project map of the proposed area to be dredged. The map MUST indicate the location of core sampling sites and the length of core samples taken.

#### SAMPLING SITE FOR REFERENCE SEDIMENT

If bioassays are required, reference sediment must be obtained from the natural marine environment. Reference sediment is sediment located near the dumpsite that is not influenced by the DISPOSAL OF DREDGED MATERIAL AT THE dumpsite. The purpose of the reference sediment is to simulate conditions at the dumpsite if PREVIOUS disposal of dredged material had not occurred. Reference sediment is compared to the proposed material to be dredged during testing and THE RESULTS ARE INTERPRETED. This will allow prediction of possible degradation within the New York Bight/Apex.

Location: Reference sediment MUST be collected outside THE MUD DUMP SITE (approximately 2.6 nautical miles southwest of the Mud Dump Site center in about 70 feet of water). Loran-C coordinates on 9960 x + y are 26910.7, 43629.2. Latitude and Longitude 40 degrees 20' 13"N, 73 degrees 52'11"W or 40 degrees 20.21'N, 73 degrees 52.19'W.

SAMPLING SITE FOR CONTROL SEDIMENT

Control sediment for the solid phase bioassay will be used to determine the health of the organisms relative to the testing conditions. When the average control mortality exceeds 10%, all solid phase bioassay testing MUST be repeated. The control sediment MUST be collected from the outer region of Milton Harbor in the City of Rye, N.Y. within the area shown on the chart on the following page.

In addition, organisms surviving the solid phase bioassay tests in the control sediment MUST be placed in sediment-free water for 24 hours to purge THEIR digestive tracts of sediment, and then immediately frozen for possible post-test bioaccumulation analysis. Organisms must be frozen for a six month period after the results of the bioassay/bioaccumulation tests have been announced in the Corps of Engineers Public Notice.

B. Physical Testing

The physical testing required for the evaluation of dredged material for ocean disposal is limited to grain size analyses and water content determinations.

CORE SAMPLES MUST BE COLLECTED TO ADEQUATELY REPRESENT THE VERTICAL AND HORIZONTAL CHARACTERISTICS OF THE MATERIAL TO BE DREDGED AND MUST BE OF SUFFICIENT VOLUME FOR CONDUCTING ALL REQUIRED ANALYSES. UNLESS VALID JUSTIFICATION FOR ANOTHER SAMPLING METHOD IS DEMONSTRATED, ALL CORE SAMPLES MUST INCLUDE SEDIMENT TO THE DEPTH OF THE PROPOSED DREDGING. IF AN ALTERNATIVE METHOD IS CONTEMPLATED, THE NEW YORK DISTRICT MUST BE CONTACTED PRIOR TO FIELD SAMPLING IN ORDER TO AVOID THE POSSIBILITY OF UNACCEPTABLE TEST RESULTS.

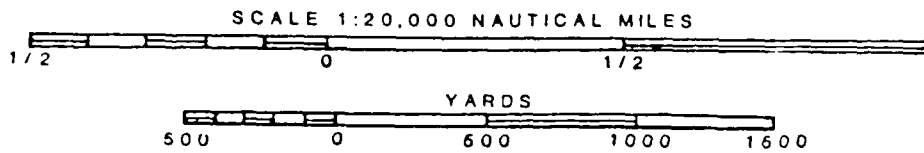
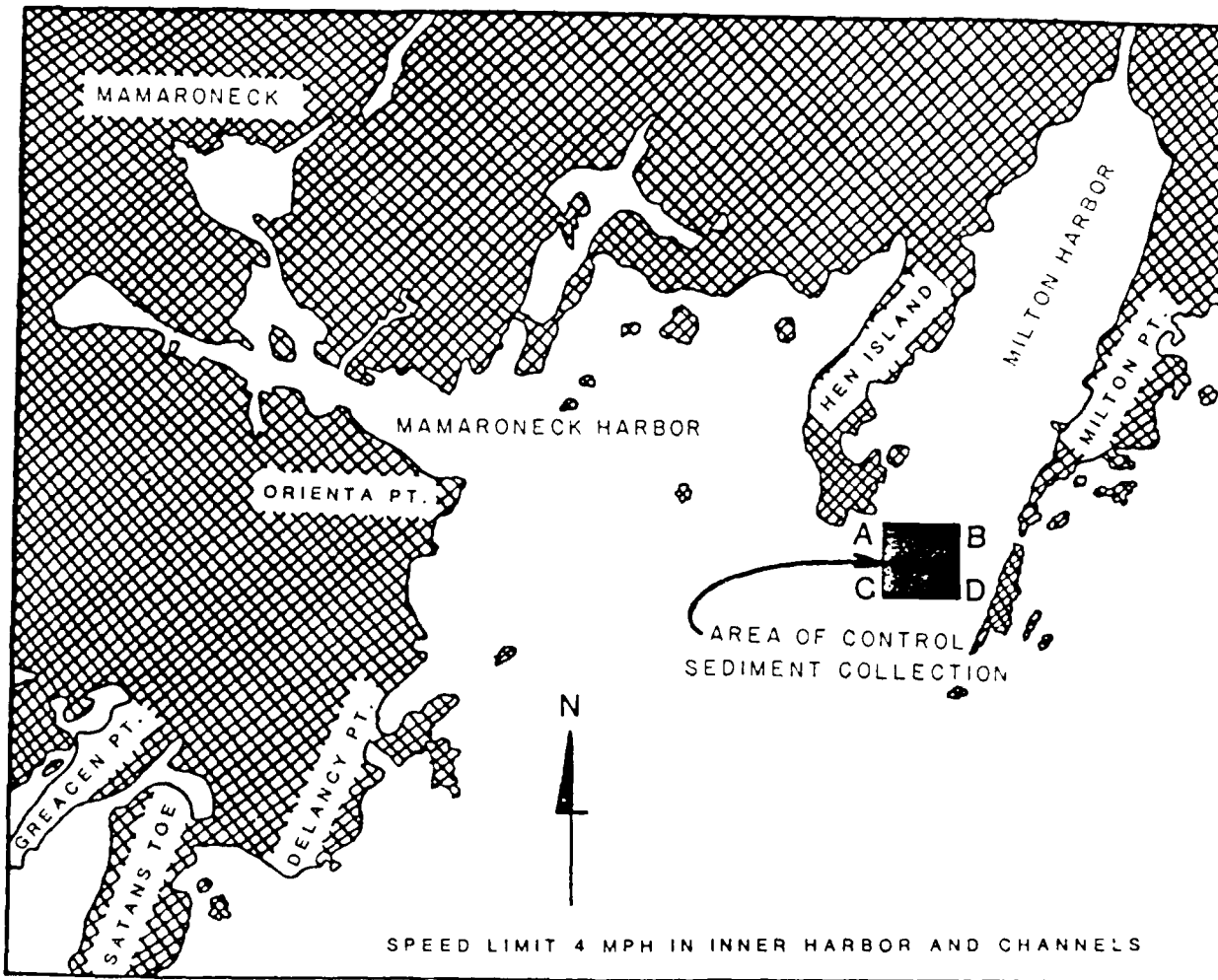
Core sediment samples MUST be visually inspected for the existence of strata formation. A grain size analysis (Folk, 1974; Guy 1969) MUST be conducted for each distinct layer observed in the material to be dredged. IN THE EVENT OF no strata formation, a minimum of three grain size analyses MUST be conducted on material obtained from three separate cores. Data MUST INCLUDE THE percentage OF sand, silt, and clay ACCORDING TO THE FOLLOWING CRITERIA:

- Sand: greater than or equal to 0.0625 mm
- Silt: less than 0.0625 mm but greater than 0.0039 mm
- Clay: less than 0.0039 mm

Folk, Robert. 1974. Petrology of Sedimentary Rocks,  
Hemphill Publishing Co., Austin, Texas.

Guy, H. P. 1969 Laboratory Theory and Methods for Sediment Analysis, Book 5:  
United States Geological Survey, 55 pp.

Chart showing area for control sediment collection



CORNER	73° 42' 19"
A	40° 56' 17"
B	73° 42' 08"
	40° 56' 17"
C	73° 42' 19"
	40° 56' 08"
D	73° 42' 08"
	40° 56' 08"

MILTON HARBOR  
RYE, NEW YORK

2a

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Grain size analysis MUST ALSO BE performed on a separate composite of the control and reference sediment used in the solid phase.

According to EPA's Ocean Dumping Criteria (Sec. 227.13(b)), the material to be dredged may be excluded from further testing if one or more of the following conditions prevail:

1. Dredged material is composed predominantly of sand, gravel, rock or any other naturally occurring bottom material with particle sizes larger than silt, AND the material is found in areas of high current or wave energy such as streams with large bed loads or coastal areas with shifting bars and channels; or
2. Dredged material is TO BE UTILIZED FOR beach nourishment or restoration and is composed predominantly of sand, gravel or shell with particle sizes compatible with material on the receiving beaches; or
3. The material proposed for dumping is substantially the same as the substrate at the proposed disposal site; AND THE PROPOSED DREDGING SITE is far removed from existing and historical sources of pollution, THEREBY providing reasonable assurance that such material has not been contaminated by pollution.

If the applicant wishes to UTILIZE one of the above exclusions, his compliance with the exclusion criteria must be demonstrated by grain size data and other pertinent, historical, or site specific information.

Other physical parameters relating to the proposed disposal operation which must be reported by the applicant are the dimensions and speed of the disposal vessel, and the duration of the disposal operation.

### C. Biological Testing

Dredged material which does not meet with the exclusions of Sec. 227.13(b) must undergo bioassay testing in accordance with Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters, second printing April 1978. The 2-phase bioassay test UTILIZES the suspended particulate and solid phases of sediment samples (including controls and replicates) to determine the effect of these phases on appropriate marine species. Testing of BOTH bioassay phases should commence within one month of sediment collection. In addition, all results of the bioassay/bioaccumulation testing MUST be submitted to this office as soon as possible to avoid the need for possible retesting due to changes in sediment characteristics as a result of discharges, shoaling or chemical spills that may have occurred IN THE INTERIM between sediment collection and the submission of testing results.

BIOASSAY TESTING OF THE LIQUID PHASE IS NOT REQUIRED. IT IS THE RESPONSIBILITY OF THE APPLICANT TO CONTACT THE COE/NYD PRIOR TO COMMENCEMENT OF TESTING TO DETERMINE THE SERIES OF TESTS REQUIRED FOR EACH INDIVIDUAL PROJECT.

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To segregate the proposed dredged material into 2 phases (liquid and suspended matter phase AND SETTLED matter phase) an elutriate separation is made by mixing dredged material with disposal site water or artificial seawater in a 1:4 (vol/vol) proportion. This mixture is subjected to vigorous 30 minute agitation and then allowed to settle undisturbed for one hour. THE SEPARATED PHASES ARE DEFINED AS FOLLOWS:

- THE SUSPENDED PARTICULATE PHASE IS THE DECANTED LIQUID AND SUSPENDED MATTER PHASE
- THE SOLID PHASE IS THE RESIDUAL SOLIDS THAT HAVE SETTLED AFTER ONE HOUR.

The sediments MUST be homogenized either by agitation on a large shaker platform or by mixing rapidly WITH a high-powered industrial-type portable mixer having a 316 stainless steel shaft and propellers (preferably a 316 stainless steel high-shear impeller).

Proposed dredged material used in the bioassays MUST be a composite of sediments collected from several sites within the proposed area to be dredged. Ocean disposal site water or artificial seawater MUST be used AS THE CONTROL WATER AS WELL AS in the elutriate separation. If ocean water is inaccessible, artificial seawater SHOULD BE PREPARED AS DESCRIBED IN STANDARD METHODS, 15TH EDITION (TABLE 80:III). THE SALINITY MUST BE  $30 \pm 2$  PPT, THE pH  $8.0 \pm 0.2$ , THE WATER TEMPERATURE  $20 \pm 2^{\circ}\text{C}$ , AND THE DO GREATER THAN 4 MG/L AT ALL TIMES.

The COE/NYD, in conjunction with EPA Region II, has designated the species contained in Table 1 as "appropriate sensitive marine organisms" to be tested in the bioassays.

### STANDARD TOXICANT

All species used by the testing laboratory in the suspended particulate phase bioassays must undergo 96 hour acute toxicity tests using the standard toxicant Sodium Lauryl Sulfate (SLS) within 30 days of the date of completion of the sample bioassay.

Laboratory grade SLS MUST be prepared immediately before use. Do not store stock solutions of SLS.

Natural seawater may not be used as dilution water for Standard Toxicant Tests. Synthetic seawater must be prepared as PREVIOUSLY described.

In general, the bioassay procedures described in the Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters, 2nd printing, April 1978, and Standard Methods, 15th Edition (pp 615-645) MUST be followed. TESTS MUST BE PERFORMED IN DUPLICATE USING 10 ORGANISMS PER REPLICATE.

The following geometric series of toxicant concentrations must be used:

a.	<u>Acartia tonsa</u>	5.0ppm,	2.5ppm,	1.3ppm,	0.6ppm,	0ppm
b.	<u>Menidia menidia</u>	5.0ppm,	2.5ppm,	1.3ppm,	0.6ppm,	0ppm
c.	<u>Mysidopsis bahia</u>	10.0ppm,	5.0ppm,	2.5ppm,	1.3ppm,	0ppm

TABLE 1. BIOASSAY TEST SPECIES &amp; BIOMASS REQUIREMENTS

Species	MINIMUM # of Organisms	Suspended Particulate Phase	Solid Phase	Static Suspended Phase Only	
				Container Size	Minimum Sample Volume
<u>Lysidopsis bahia</u> (crustacean)	20	YES	NO	1 liter crystallizing dish	800 ml
<u>lenidia menidia</u> (finfish)	20	YES	NO	37 liter tank	20 liters
<u>cartia tonsa</u> (zooplankton)	20	YES	NO	200 ml crystallizing dish	160 ml
<u>tereis virens</u> (sand worm)	20	NO	YES	37 liter tank	20 liters
<u>mercenaria mercenaria</u> (clam)	20	NO	YES	37 liter tank	20 liters
<u>alaemonetes pugio</u> (crustacean)	20	NO	YES	37 liter tank	20 liters

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IF THE HIGHEST CONCENTRATION INDICATED ABOVE DOES NOT RESULT IN 50% MORTALITY AFTER 96 HOURS, PROGRESSIVELY HIGHER CONCENTRATIONS MUST BE USED UNTIL THIS MORTALITY RATE IS OBTAINED.

CONTROL MORTALITY MUST NOT EXCEED 10% OR THE RESULTS ARE DEEMED UNACCEPTABLE AND THE TEST MUST BE REPEATED.

A summary of the standard toxicant test must be included in each Laboratory Report submitted to the COE/NYD and MUST include the following information (one sheet per organism):

- a. Test organism species, source of specimens
- b. Test start date, test finish date
- c. Brand name of artificial seawater mix
- d. Toxicant brand name and grade
- e. The number of live organisms  
at 0,4,8,24,48,72, and 96 hours
- f. Salinity, temperature, pH and DO values  
at 0,24,48,72, and 96 hours
- g. Method of calculating LC50
- h. LC50 values with 95% Confidence Intervals

### SCHEDULE I LIQUID PHASE ASSAY

THIS TESTING PROCEDURE IS NO LONGER REQUIRED.

### SCHEDULE II SUSPENDED PARTICULATE ASSAY

A single suspended particulate phase sample refers to one homogenized suspension which undergoes assays WITH three different species, Acartia tonsa, Mysidopsis bahia, and Menidia menidia. All procedures, unless authorized in writing, MUST conform to the guidelines established in the publication Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters., Second printing, April 1978. During the suspended phase assays, assessments of sublethal effects must also be made. BIOASSAYS MUST BE PERFORMED AS FOLLOWS:

#### IIa. SUSPENDED PARTICULATE PHASE ASSAY USING Acartia tonsa

EACH SAMPLE MUST BE SUB-SAMPLED ACCORDINGLY, USING A MINIMUM OF 20 SPECIMENS PER REPLICATE ASSAY:

1. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% CONTROL WATER.
2. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% SUSPENDED PARTICULATE PHASE.
3. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 50% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.

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4. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 10% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.

DURATION OF ASSAYS SHOULD BE A MINIMUM OF 96 HOURS WITH ASSESSMENT OF MORTALITY AND ALSO ANY SUBLETHAL EFFECTS TO BE MADE AND REPORTED AT 0 HOURS, 4, 8, 24, 48, 72 AND 96 HOURS. SUBLETHAL EFFECTS ARE DEFINED AS ANY OBVIOUS PHYSICAL OR BEHAVIORAL ANOMALIES. THESE OBSERVATIONS (INCLUDING OBSERVING NOTHING OUT OF THE ORDINARY) MUST BE REPORTED ALONG WITH ASSESSMENTS OF MORTALITY.

IIb. SUSPENDED PARTICULATE PHASE ASSAY USING *Mysidopsis bahia*

EACH SAMPLE MUST BE SUB-SAMPLED ACCORDINGLY, USING A MINIMUM OF 20 SPECIMENS PER REPLICATE ASSAY:

1. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% CONTROL WATER.
2. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% SUSPENDED PARTICULATE PHASE.
3. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 50% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.
4. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 10% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.

DURATION OF ASSAYS SHOULD BE A MINIMUM OF 96 HOURS WITH ASSESSMENT OF MORTALITY AND ALSO ANY SUBLETHAL EFFECTS TO BE MADE AND REPORTED AT 0 HOURS, 4, 8, 24, 48, 72 AND 96 HOURS. SUBLETHAL EFFECTS ARE DEFINED AS ANY OBVIOUS PHYSICAL OR BEHAVIORAL ANOMALIES. THESE OBSERVATIONS (INCLUDING OBSERVING NOTHING OUT OF THE ORDINARY) MUST BE REPORTED ALONG WITH ASSESSMENTS OF MORTALITY.

IIc. SUSPENDED PARTICULATE PHASE USING *Menidia menidia*

EACH SAMPLE MUST BE SUB-SAMPLED ACCORDINGLY, USING A MINIMUM OF 20 SPECIMENS PER REPLICATE.

1. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% CONTROL WATER.
2. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 100% SUSPENDED PARTICULATE PHASE.
3. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 50% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.
4. INDIVIDUAL ASSAYS PERFORMED IN TRIPLICATE ON 10% SUSPENDED PARTICULATE PHASE, THE BALANCE CONSISTING OF CONTROL WATER.

DURATION OF ASSAYS IS TO BE A MINIMUM OF 96 HOURS WITH ASSESSMENT OF MORTALITY AND ALSO ANY SUBLETHAL EFFECTS TO BE MADE AND REPORTED AT 0, 4, 8, 24, 48, 72 AND 96 HOURS. SUBLETHAL EFFECTS ARE DEFINED AS ANY OBVIOUS PHYSICAL OR BEHAVIORAL ANOMALIES. THESE OBSERVATIONS (INCLUDING OBSERVING NOTHING OUT OF THE ORDINARY) MUST BE REPORTED ALONG WITH ASSESSMENTS OF MORTALITY.

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Schedule II for Suspended Particulate Phase Bioassays outlines the minimum number of concentrations at which assays must be PERFORMED. If highly toxic conditions exist such that at the 10% concentration there is greater than 50% mortality, further dilution must be made in order to attain a greater than 50% survival, so an LC50 may be determined by interpolation. These dilutions, if necessary, must also be done in triplicate.

### SCHEDULE III SOLID PHASE ASSAY

A single solid phase sample refers to one homogenized sediment-slurry which undergoes assays by the three different species described below in IIIa, IIIb and IIIc.

All procedures, unless authorized in writing, are to conform to the guidelines established in the publication "Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters" second printing, April 1978.

AS SPECIFIED IN THE ABOVE REFERENCED PUBLICATION, THE FLOW-THROUGH SYSTEM MUST PROVIDE 6 CHANGES OF WATER PER 24 HOURS. The flow injection MUST be directed downward at 2" below the surface in order to achieve good mixing without disturbing the layer of sediment at the bottom.

REPLICATES FOR TEST, REFERENCE AND CONTROL TREATMENTS MUST BE RUN IN SEPARATE AQUARIA; HOWEVER, SPECIES MAY BE COMBINED IN AQUARIA IF ORGANISMS SHOW COMPATIBILITY IN THE NATURAL ENVIRONMENT. THE LABORATORY MUST ENSURE THAT MASS LOADINGS OF CONTROLS AND REFERENCES ARE SIMILAR TO THAT OF TEST SEDIMENT, AND THAT MASS LOADINGS OF INDIVIDUAL TANKS ARE CONDUCTIVE TO SURVIVAL OF THE ORGANISMS. IN ADDITION, LABORATORIES MUST ENSURE THAT AN ADEQUATE AMOUNT OF ANIMAL TISSUE IS AVAILABLE TO CONDUCT ALL REQUIRED BIOACCUMULATION ANALYSES AS DESCRIBED IN THE APPENDIX.

Pretest analyses for contaminants of concern in randomly selected organisms MUST BE PERFORMED. A MINIMUM OF twenty organisms MUST BE USED in order to obtain tissue for 3 aliquots PER SPECIES, resulting in a total of 9 aliquots. Each aliquot MUST be analyzed for all FIVE contaminants and the results included with the test report. The constituent levels analyzed from organism tissue MUST NOT EXCEED the specified detection limits for any parameter, with the exception of petroleum hydrocarbons. Since the analysis for total Petroleum Hydrocarbons includes the natural body lipids of the organism, the maximum limit for the constituent MUST NOT exceed 1.0 ppm.

Treatment tanks MUST contain the following layers of sediment FOR SOLID PHASE TESTING:

- |                           |   |  |
|---------------------------|---|--|
| Test treatment tanks      | - | 30mm layer of reference sediment plus a 15mm layer of the dredged material to be tested placed on top. |
| Reference treatment tanks | - | 45mm layer of reference sediment   |
| Control treatment tanks   | - | 45mm layer of control sediment   |

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IF THE MORTALITY RATE OF THE TEST ORGANISMS IS EXCESSIVE, FROZEN TISSUE ANALYSIS FROM THE CONTROL SOLID PHASE MAY BE REQUIRED. THE REQUIREMENT FOR THIS TEST, ALONG WITH THE NUMBER OF SAMPLES AND QUANTITIES SPECIFIED, IS THE OPTION OF THE NEW YORK DISTRICT CORPS OF ENGINEERS.

### IIIa. Solid Phase Assays Using Palaemonetes pugio

Each sample MUST be sub-sampled accordingly, using a minimum of 20 organisms per replicate:

1. Three replicate assays MUST BE performed using the specified control sediment.
2. Five replicate assays MUST BE performed using the specified reference sediment.
3. Five replicate assays MUST BE performed using a homogenized solid phase sample.

### IIIb. Solid Phase Assays Using Mercenaria mercenaria

Each sample MUST be sub-sampled accordingly, using a minimum of 20 organisms per replicate:

1. Three replicate assays MUST BE performed using the specified control sediment.
2. Five replicate assays MUST BE performed using the specified reference sediment.
3. Five replicate assays MUST BE performed using a homogenized solid phase.

### IIIc. Solid Phase Assays Using Nereis virens

Each sample MUST be sub-sampled accordingly, using a minimum of 20 organisms per replicate:

1. Three replicate bioassays MUST BE performed using the specified control sediment.
2. Five replicate bioassays MUST BE performed using the specified reference sediment.
3. Five replicate bioassays MUST BE performed using a homogenized solid phase sample.

The solid phase assays MUST continue for 10 days, during which time daily records must be kept of salinity, temperature, DO, obvious mortalities and any sublethal effects. Formation of tubes or burrows and any physical or behavioral abnormalities MUST also be recorded. These daily records must be REPORTED BY THE TESTING LABORATORY AND SUBMITTED BY THE APPLICANT. Organisms should be fed on a daily basis AT A RATE OF approximately 1% of the total

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weight of all animals in each tank. Caution should be taken not to overfeed organisms; if excess food appears in the tank, the percentage of food should be reduced.

ALL ORGANISMS SURVIVING THE SOLID PHASE MUST BE PLACED IN SEDIMENT-FREE WATER FOR 24 HOURS TO PURGE THEIR DIGESTIVE TRACTS OF SEDIMENT. Organisms surviving the solid phase in both the test and reference treatment should then be saved for bioaccumulation analyses. Organisms surviving the control treatment will not be analyzed unless requested by the COE/NYD. Control organisms MUST be kept frozen at minus 20 degrees celsius FOR SIX MONTHS FOLLOWING THE ANNOUNCEMENT OF THE BIOASSAY/BIOACCUMULATION TESTS IN THE COE PUBLIC NOTICE.

### D. Chemical Testing

If dredged material does not meet with the exclusions of Sec. 227.13(b), EPA Region II, under the authority of Section 225.2(b), has requested that chemical analysis of site water MUST be conducted. These analyses MUST be performed upon THREE separate samples of the LIQUID PHASE ELutriate PREPARED USING DREDGING SITE WATER. A SINGLE ANALYSIS MUST BE PERFORMED ON EACH SUB-SAMPLE. All procedures, unless authorized in writing, MUST conform to the guidelines established in the publication Ecological Evaluation of Proposed Discharge Material Into Ocean Waters, Second printing, April 1978. THE CONTAMINANTS TO BE TESTED AND THE REQUIRED DETECTION LIMITS ARE SUMMARIZED BELOW:

<u>Contaminants</u>	<u>Required Detection Limits</u>
1. Hg (MERCURY)	0.2 ug/liter
2. Cd (CADMIUM)	0.1 ug/liter
3. PCB's	0.1 ug/liter
4. Petroleum Hydrocarbons	50.0 ug/liter
5. DDT	0.05 ug/liter

The above list represents THE minimum number of contaminants to be tested IN THE CHEMICAL ANALYSIS OF THE ELutriate AND SITE WATER. This list may be modified by additions based on data from A-2. If the applicant has knowledge of nearby sources of contamination which may be affecting the sediments to be dredged, he must undertake the testing of those additional chemical contaminants and report those data along with the results of the aforementioned contaminants.

### E. Bioaccumulation Analysis

BIOACCUMULATION ANALYSES MUST BE PERFORMED FOR Hg, Cd, PCBs, PETROLEUM



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HYDROCARBONS, AND DDT ON ALL TEST ORGANISMS SURVIVING THE 10 DAY SOLID PHASE EXPOSURE. THE REQUIRED DETECTION LIMITS FOR EACH CONSTITUENT ARE SUMMARIZED BELOW:

	<u>CONSTITUENT</u>	<u>REQUIRED DETECTION LIMIT</u>
1.	Hq	0.20 MG/KG
2.	Cd	0.25 MG/KG
3.	PCBs	0.04 MG/KG
4.	PETROLEUM HYDROCARBONS	0.10 MG/KG
5.	DDT	0.02 MG/KG

THE COE/NYD RESERVES THE RIGHT TO MODIFY THIS LIST IN ACCORDANCE WITH THE CRITERIA SPECIFIED IN SECTION A-2. TEST ORGANISMS MUST BE Palaemonetes pugio, Nereis virens, AND Mercenaria mercenaria.

THE PROCEDURES FOR SEDIMENT BIOACCUMULATION ANALYSES PROVIDED IN PROCEDURES FOR HANDLING AND CHEMICAL ANALYSIS OF SEDIMENT AND WATER SAMPLES MUST BE FOLLOWED. METHODS FOR TISSUE ANALYSES ARE PROVIDED IN INTERIM METHODS FOR THE SAMPLING AND ANALYSIS OF PRIORITY POLLUTANTS IN SEDIMENTS AND FISH TISSUE; SECTIONS OF THIS PUBLICATION ARE REPRINTED IN THE APPENDIX OF THIS MANUAL. IT SHOULD BE STRESSED THAT THE APPENDIX CONTAINS PROCEDURES FOR Hq, Cd, PCBs, AND DDT, AS WELL AS OTHER CONSTITUENTS WHICH MAY BE REQUIRED ON A CASE-BY-CASE BASIS. AT PRESENT, THERE IS NO OFFICIAL PROCEDURE FOR PETROLEUM HYDROCARBON ANALYSIS.

The need for suitable tissue sample size MUST BE STRESSED in order to obtain the detection limits listed. A separate analysis must be conducted for each chemical constituent, for each individual replicate, and for each of the animal species in both test and reference treatments. In addition, 3 sub-samples of the homogenate from one of the five replicates in the test treatments for each of THE 3 species MUST also be analyzed for the 5 chemical constituents in order to estimate the precision of the analytical method. These 3 sub-samples MUST be redigested and re-extracted in order to determine the % recovery of the original extraction. These re-extractions need be performed only on the tissues from the test phase. All data generated from these procedures MUST be reported by the applicant. Other constituents (Implementation Manual p G8) MAY be required for analyses whenever the District Engineer and Regional Administrator have reason to believe THAT they may be WARRANTED.

F. Bulk Sediment Analysis

Bulk sediment analyses MUST be conducted on sediment samples collected at the sites where grain size analyses are performed. Core sediment samples MUST be visually inspected for the existence of strata formation AND A bulk sediment analysis MUST be conducted for each distinct layer observed in the material to be dredged. Should it be observed that there is no strata formation, a minimum of three bulk sediment analyses MUST be conducted on material obtained from three separate cores. The constituents to be tested ARE copper, zinc, nickel, percent moisture and total organic carbon as measured by loss on ignition. The required detection limits for heavy metals listed IS 1.0 ppm. All procedures, unless authorized in writing, MUST conform to procedures established in the EPA/COE publication, Procedures for Handling and Chemical Analysis of Sediment and Water Samples, May 1981.

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TECHNICALLY, bulk sediment analyses are not REQUIRED AS PART of the Ocean Dumping Criteria. However, bulk sediment analyses MUST be conducted for the purposes of determining and establishing a chemical status of the sediment.

### G. LONG ISLAND SOUND DISPOSAL TESTING REQUIREMENTS

DISPOSAL OF DREDGED MATERIAL IN LONG ISLAND SOUND HAS BEEN THE SUBJECT OF INCREASED ENVIRONMENTAL AWARENESS AND PUBLIC CONCERN. THE NUMBER OF DREDGED MATERIAL DISPOSAL SITES IN WESTERN LONG ISLAND SOUND HAS BEEN REDUCED TO ONE SITE (WLIS III), AND THE NUMBER OF FEDERALLY AUTHORIZED CHANNELS THAT COULD POTENTIALLY UTILIZE THIS SITE HAS BEEN CURTAILED. AT PRESENT, ALL NEW YORK HARBORS ELIGIBLE TO UTILIZE THIS DISPOSAL SITE MUST BE LOCATED EAST OF THE THROGS NECK BRIDGE. A LIST OF THE ELIGIBLE HARBORS IS PRESENTED BELOW:

1. PORT CHESTER HARBOR
2. MILTON HARBOR
3. MAMARONECK HARBOR
4. ECHO BAY HARBOR
5. NEW ROCHELLE HARBOR
6. EASTCHESTER CREEK
7. LITTLE NECK BAY
8. MANHASSET BAY
9. HEMPSTEAD HARBOR
10. GLEN COVE CREEK AND HARBOR
11. HUNTINGTON HARBOR
12. NORTHPORT HARBOR

ALL PERMIT APPLICANTS ARE REQUIRED TO HAVE THEIR SAMPLING PLAN APPROVED BY COE/NYD PRIOR TO IMPLEMENTATION. THE MAGNITUDE OF REQUIRED TESTING WILL BE DETERMINED ON A CASE-BY-CASE BASIS. ALL OF THE PROCEDURES PRESENTED IN SECTIONS A-F OF THIS MANUAL MAY BE APPLIED TO LONG ISLAND SOUND DREDGING PROJECTS, IF SUCH TESTING IS DEEMED NECESSARY.

PRIOR TO COMMENCEMENT OF TESTING, THE APPLICANT MUST INFORM THE COE/NYD WHICH LABORATORY WILL BE PERFORMING THE BIOLOGICAL AND ANALYTICAL TESTING (SEE PAGE iii AND SECTION I).

### H. Laboratory Quality Assurance Program

Concern has been expressed by both the COE/NYD and EPA staffs ABOUT the reproducibility of data submitted by permit applicants. To insure that data submitted are reliable and accurate, the COE/NYD in conjunction with EPA Region II, has developed the following quality control program.

ALL BIOASSAYS MUST BE PERFORMED AT 20° C ( $\pm 2^{\circ}$ ) IN EITHER NATURAL SEAWATER OR A SYNTHETIC SEAWATER ADJUSTED TO 30 PARTS PER THOUSAND SALINITY. IF A SYNTHETIC SEAWATER IS USED, THE MIXTURE MUST BE ALLOWED TO AGE SUFFICIENTLY PRIOR TO USE. IF NATURAL SEAWATER IS USED, THE INFLUENT WATER MUST BE CHECKED AT THE START AND FINISH OF EACH TEST FOR CADMIUM, MERCURY, PCBS, DDT, AND PETROLEUM HYDROCARBONS TO ASCERTAIN THAT THE CONCENTRATION OF THESE SUBSTANCES IN THE WATER IS NOT IMPACTING THE TESTS.

REFERENCE AND CONTROL SEDIMENTS MUST BE COLLECTED FROM THE LOCALITY SPECIFIED IN SECTION A, AND GRAIN SIZE ANALYSES MUST BE PERFORMED.

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CONTROL BIOASSAYS MUST MAINTAIN AN AVERAGE OF 90% SURVIVAL RATE AMONG THE REPLICATES FOR EACH SPECIES TESTED EXCLUDING ZOOPLANKTON. CONTROL MORTALITIES OF 20% ARE ACCEPTABLE IN ZOOPLANKTON BIOASSAYS. FAILURE TO MAINTAIN THE SURVIVAL RATES WILL INVALIDATE THE TESTING PROCEDURES AND REQUIRE RETESTING OF THE CONTROL, REFERENCE, AND TEST SAMPLES.

ALL LABORATORIES PROVIDING ANALYTICAL SERVICES TO PERMIT APPLICANTS MUST PERFORM TESTING IN ACCORDANCE WITH THE FOLLOWING SPECIFICATIONS:

1. Standard toxicant tests must be performed on species used in the suspended particulate phases.
2. Any laboratory employed for the purposes of performing the analyses specified herein MUST maintain a viable analytical quality control program, which shall include:
  - a. use of COE/EPA analytical test procedures as recommended in the EPA manual PROCEDURES FOR HANDLING AND CHEMICAL ANALYSIS OF SEDIMENT AND WATER SAMPLES (TECHNICAL REPORT EPA/CEB1-1; MAY 1981).\*
  - b. use of sample preservation techniques and holding time specified in the EPA manual Methods for Chemical Analysis of Water and Wastes (EPA-600/4-79-020; Revised March 1983).\*
  - c. routine use and documentation of intra-laboratory quality control practices as recommended in the EPA manual Handbook for Analytical Quality Control in Water and Wastewater Laboratories.\* These practices must include use and documentation of internal quality control samples.
3. The laboratory facilities are subject to periodic inspection by COE/NYD AND EPA PERSONNEL. ORIGINAL COPIES OF DATA, RECORDS, AND QUALITY CONTROL INFORMATION CONCERNING SEDIMENT TESTING FOR A CLIENT FOR A DEPARTMENT OF THE ARMY PERMIT MUST BE MAINTAINED FOR A PERIOD OF AT LEAST THREE (3) YEARS AND MUST BE AVAILABLE DURING LABORATORY INSPECTIONS.
4. The COE/NYD may require analysis of quality control samples by any laboratory for THE purpose of DETERMINING compliance with its analytical requirements. Such samples shall be limited to four (4) per calendar year. Upon request, the laboratory shall provide the New York District with the analytical results from such quality control samples.
5. The COE/NYD will periodically inspect laboratories for the purpose of evaluating their capabilities in performing the requirements specified in the Guidance Manual.

\*Copies of these manuals may be obtained from the Quality Assurance Office, EPA Region II, Edison, NJ - (201) 321-6645.

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6. Upon submission of the test results to the COE/NYD, the laboratory MUST concurrently submit their current Quality Assurance MANUAL (QAM) for review by the District. The QAM MUST include:
  - a. A LIST of all analytical equipment (make, model and year) and devices used in the biological and chemical work, laboratory calibration methods, precision and accuracy standards, number of times standards are checked, maintenance schedules, record-keeping methods, personnel responsibilities, and source of test animals.
  - b. Labeling system employed to ensure proper tracking of samples from collection through analysis to listing in THE final report.
  - c. Laboratory analytical techniques, referencing manual, method number and/or page, and quality control procedures outlined step by step in flow-chart format.

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### I. Final Note on Laboratory Selection

The COE/NYD is aware that a variety of laboratories in the New York-New Jersey area, as well as throughout the country, have expressed interest in performing the required testing for ocean disposal. These laboratories vary considerably in price and quality of performance. Some laboratories have had more experience than others in these particular analyses. At this time there is no EPA certification for the analytical testing laboratories FOR BIOASSAY/BIOACCUMULATION TESTING within the NYD.

The applicant MUST ASCERTAIN THAT THE LABORATORY SELECTED HAS ACCEPTABLE PERSONNEL, EQUIPMENT AND FACILITIES TO MEET THE requirements contained herein AS WELL AS in the EPA/COE Manual for Ocean Disposal. No requirement will be waived for an applicant because of failure of the laboratory to comply with the guidelines set forth here and in the Implementation Manual.

COE/NYD personnel are available to answer questions which may arise in the course of the testing process. Questions may be addressed to:

U. S. Army Corps of Engineers  
New York District  
Regulatory Branch  
Water Quality Compliance Section  
26 Federal Plaza  
New York, New York 10278-0090

Phone: (212) 264-5620

Additional copies of this Guidance Manual may be obtained at the above address.

Copies of the EPA/COE Implementation Manual Criterion Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Water and Implementation Manual for Section 103 of the Public Law 92-532 (Marine Protection Research and Sanctuaries Act of 1972), July 1977 (Second Printing April 1978) may be obtained by writing to:

Environmental Effects Laboratory  
U.S. Army Engineer Waterways Experiment Station  
P.O. Box 631  
Vicksburg, Mississippi 39180-0631

ATTN: Publications Office

or by calling - (601) 636-3111 - Publications Office

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APPENDIX

PROCEDURES FOR TISSUE BIOACCUMULATION  
ANALYSIS

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INTRODUCTION

THE ANALYTICAL PROCEDURES DESCRIBED IN THIS APPENDIX ARE TAKEN FROM AN EPA REPORT ENTITLED INTERIM METHODS FOR THE SAMPLING AND ANALYSIS OF PRIORITY POLLUTANTS IN SEDIMENTS AND FISH TISSUES (AUGUST 1977; REVISED OCTOBER 1980). MINOR MODIFICATIONS HAVE BEEN MADE TO THAT TEXT IN ORDER TO MATCH THE TERMINOLOGY USED IN THIS MANUAL, BUT THE ANALYTICAL METHODS REMAIN UNCHANGED.

AT PRESENT, TISSUE BIOACCUMULATION TESTS ARE REQUIRED FOR MERCURY, CADMIUM, PCBs, DDT AND PETROLEUM HYDROCARBONS, UNLESS OTHERWISE INDICATED BY ENVIRONMENTAL CONDITIONS ON A CASE-BY-CASE BASIS. THE REQUIRED METHODS FOR FOUR OF THESE CONSTITUENTS (Hg, Cd, PCBs, DDT) ARE CONTAINED IN THIS APPENDIX; HOWEVER, BECAUSE THERE PRESENTLY IS NO EPA AUTHORIZED TISSUE BIOACCUMULATION PROCEDURE FOR PETROLEUM HYDROCARBONS, THE APPLICANT MAY UTILIZE ANY APPROPRIATE METHOD. IT IS THE APPLICANT'S RESPONSIBILITY TO CONTACT THE NEW YORK DISTRICT CORPS OF ENGINEERS PRIOR TO COMMENCEMENT OF TESTING TO ASCERTAIN THAT THEIR METHOD IS INDEED APPROPRIATE.

ANALYSIS OF TISSUE FOR CHLORINATED PESTICIDES  
AND POLYCHLORINATED BIPHENYLS

1. SCOPE

1.1 THE CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBs) LISTED IN TABLE A ARE EXTRACTED FROM TISSUES USING EITHER METHOD A OR B AS DESCRIBED BELOW. METHOD A EMPLOYS A BLENDER, WHEREAS A TISSUMIZER OR THE EQUIVALENT IS REQUIRED FOR METHOD B. EITHER PROCEDURE RESULTS IN AN EXTRACT THAT CAN BE INCORPORATED DIRECTLY INTO THE APPROVED PROCEDURES (1) FOR PESTICIDES OR PCBs AS CITED IN THE FEDERAL REGISTER (2).

2. SPECIAL APPARATUS AND MATERIALS

2.1 METHOD A ONLY

2.1.1 BLENDER, HIGH-SPEED - WARING BLENDER, COURDOS, OMNI-MIXER, OR EQUIVALENT. EXPLOSION PROOF MODEL RECOMMENDED. QUART CONTAINER IS SUITABLE SIZE FOR ROUTINE USE.

2.1.2 BUCHNER FUNNEL - PORCELAIN, 12-CM.

2.1.3 FILTER PAPER - 110 MM SHARKSKIN CIRCLES.

2.1.4 FLASK, VACUUM FILTRATION - 500 ML.

2.2 METHOD B ONLY

2.2.1 TISSUMIZER SDT-182EN (AVAILABLE FROM TEKMAR COMPANY, P.O. BOX 37207, CINCINNATI, OHIO 45222), OR EQUIVALENT.

2.2.2 CENTRIFUGE - CAPABLE OF HANDLING 100 ML CENTRIFUGE TUBES.

2.3 METHODS A & B

2.3.1 KUDERNA-DANISH CONCENTRATOR - 500 ML, WITH 10 ML GRADUATED RECEIVER AND 3-BALL SNYDER COLUMN.

2.3.2 CHROMATOGRAPHIC COLUMN - PYREX, 20 MM ID x APPROXIMATELY 400 MM LONG, WITH COARSE FRITTED PLATE ON BOTTOM.

3. PROCEDURES

3.1 METHOD A

3.1.1 WEIGH A 25 TO 50 G PORTION OF FROZEN, GROUND TISSUE. PLACE IN A HIGH-SPEED BLENDER. ADD 100 G ANHYDROUS  $\text{Na}_2\text{SO}_4$  TO COMBINE WITH THE WATER AND TO DISINTEGRATE THE SAMPLE. ALTERNATELY, BLEND AND MIX WITH A SPATULA UNTIL THE SAMPLE AND SODIUM SULFATE ARE WELL MIXED. SCRAPE DOWN THE SIDE OF THE BLENDER JAR AND BREAK UP THE CAKED



TABLE A

PRIORITY POLLUTANTS ANALYZED BY SOXHLET EXTRACTIONPESTICIDES

ALDRIN	DDD	ENDOSULFAN SULFATE
a-BHC	DDE	ENDRIN
b-BHC	DDT	ENDRIN ALDEHYDE
d-BHC	DIELDRIN	HEPTACHLOR
q-BHC	ENDOSULFAN - I	HEPTACHLOR EPOXIDE
CHLORDANE	ENDOSULFAN - II	TOXAPHENE

PCBs

AROCLOR 1016	AROCLOR 1242	AROCLOR 1254
AROCLOR 1221	AROCLOR 1243	AROCLOR 1260
AROCLOR 1232		

NON-POLAR NEUTRALS

ACENAPHTHYLENE	1,3-DICHLOROBENZENE	BIS (2-ETHYLHEXYL) PHTHALATE
ACENAPHTHENE	1,4-DICHLOROBENZENE	BENZO (a) ANTHRACENE
ISOPHORONE	HEXACHLORETHANE	BENZO (b) FLUORANTHENE
FLUROENE	1,2-DICHLOROBENZENE	BENZO (k) FLUORANTHENE
PHENANTHRENE	HEXACHLOROBUTADIENE	BENZO (a) PYRENE
ANTHRACENE	1,2,4-TRICHLOROBENZENE	INDENO (1,2,3-cd) PYRENE
DIMETHYLPHTHALATE	2,6-DINITROTOLUENE	DIBENZO (a,h) ANTHRACENE
DIETHYLPHTHALATE	HEXACHLOROBENZENE	BENZO (ghi) PERYLENE
FLUORANTHENE	4-BROMOPHENYL PHENYL ETHER	4-CHLOROPHENYL PHENYL ETHER
PYRENE	BIS (2-CHLOROETHOXY) METHANE	2,3,7,8-TETRACHLORODIBENZO-f DIOXIN
NAPHTHALENE	2-CHLORONAPHTHALENE	DI-N-BUTYLPHTHALATE
CHRYSENE		BUTYL BENZYLPHTHALATE

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MATERIAL WITH THE SPATULA. ADD 150 ML OF HEXANE AND BLEND AT HIGH SPEED FOR 2 MIN.

- 3.1.2 DECANT THE HEXANE SUPERNATANT THROUGH A 12-CM BUCHNER FILTER WITH TWO SHARKSKIN PAPERS INTO A 500-ML SUCTION FLASK. SCRAPE DOWN THE SIDES OF THE BLENDER JAR AND BREAK UP THE CAKED MATERIAL WITH THE SPATULA. REEXTRACT THE RESIDUE IN THE BLENDER JAR WITH TWO 100 ML PORTIONS OF HEXANE, BLENDING 3 MIN. EACH TIME. (AFTER ONE MIN. OF BLENDING, STOP THE BLENDER, SCRAPE THE MATERIAL FROM THE SIDES OF THE BLENDER JAR, AND BREAK UP THE CAKED MATERIAL BETWEEN EXTRACTIONS.)
- 3.1.3 DECANT THE HEXANE SUPERNATANTS THROUGH THE BUCHNER AND COMBINE WITH THE FIRST EXTRACT. AFTER THE LAST BLENDING, TRANSFER THE RESIDUE FROM THE BLENDER JAR TO THE BUCHNER, RINSING THE BLENDER JAR AND MATERIAL IN THE BUCHNER WITH THREE 25 TO 50 ML PORTIONS OF HEXANE. IMMEDIATELY AFTER THE LAST RINSE, PRESS THE RESIDUE IN THE BUCHNER WITH THE BOTTOM OF A CLEAN BEAKER TO FORCE OUT THE REMAINING HEXANE.
- 3.1.4 POUR THE COMBINED EXTRACTS AND RINSES THROUGH A COLUMN OF ANHYDROUS  $\text{Na}_2\text{SO}_4$ , 20 MM X 100 MM, AND COLLECT THE ELUTRIATE IN A 500 ML KUDERNA-DANISH CONCENTRATOR. WASH THE FLASK AND THE COLUMN WITH SMALL PORTIONS OF HEXANE AND CONCENTRATE THE EXTRACT TO 10 ML.

### 3.2 METHOD B

- 3.2.1 WEIGH 20.0G OF FROZEN, GROUND TISSUE AND PLACE IN A 100-ML CENTRIFUGE TUBE. ADD 20 ML OF HEXANE AND INSERT THE TISSUMIZER INTO THE SAMPLE. TURN ON THE TISSUMIZER AND DISPERSE THE TISSUE IN THE SOLVENT FOR 1 MINUTE. CENTRIFUGE AND DECANT THE SOLVENT THROUGH A COLUMN OF ANHYDROUS  $\text{Na}_2\text{SO}_4$ , 20 MM X 100 MM, AND COLLECT THE ELUTRIATE IN A 500-ML KUDERNA-DANISH CONCENTRATOR.
- 3.2.2 REPEAT THE DISPERSION TWICE USING A 20-ML ALIQUOT EACH TIME, COMBINING ALL DRIED PORTIONS OF SOLVENT IN THE CONCENTRATOR. RINSE THE TISSUMIZER AND THE COLUMN WITH SMALL PORTIONS OF HEXANE AND CONCENTRATE THE EXTRACT TO 10 ML.

### 3.3 CLEANUP AND ANALYSIS

- 3.3.1 UNLESS PRIOR EXPERIENCE INDICATES THAT THE TISSUE FAT CONTENT FOR A PARTICULAR SPECIES IS LOW (LESS THAN 3G PER EXTRACT), THE HEXANE/ACETONITRILE CLEANUP PROCEDURES DESCRIBED IN THE REFERENCE METHODS MUST BE FOLLOWED. IN ALL CASES, FLORISIL COLUMN CHROMATOGRAPHY MUST BE USED TO CLEAN UP THE EXTRACTS PRIOR TO GAS CHROMATOGRAPHY (1). AN

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ELECTRON CAPTURE DETECTOR IS USED FOR FINAL MEASUREMENT, AND RESULTS ARE CALCULATED IN UG/KG. IDENTIFICATIONS CAN BE CONFIRMED BY GC/MS TECHNIQUES AS DESCRIBED IN APPENDIX II OF THE FEDERAL REGISTER (3).

### 3.4 QUALITY CONTROL

3.4.1 STANDARD QUALITY ASSURANCE PROTOCOLS MUST BE EMPLOYED FOR BLANKS, DUPLICATES, AND DOSED SAMPLES AS DESCRIBED IN THE "ANALYTICAL QUALITY CONTROL HANDBOOK" (4).

3.4.2 DOSE TISSUE SAMPLE ALIQUOTS BY INJECTING MINIMUM AMOUNTS (20UL TOTAL) OF CONCENTRATED PESTICIDE OR PCB SOLUTIONS INTO THE SOLID SUBSAMPLE 10 TO 15 MINUTES BEFORE EXTRACTION.

### 4. REPORTING OF DATA

REPORT RESULTS IN UG/KG ON A WET TISSUE BASIS. REPORT ALL QUALITY CONTROL DATA WITH THE ANALYTICAL RESULTS FOR THE SAMPLES.

ANALYSIS OF TISSUE FOR MERCURY

1. SCOPE AND APPLICATION

- 1.1 THIS METHOD IS USED FOR DETERMINATION OF TOTAL MERCURY (ORGANIC AND INORGANIC) IN ANIMAL TISSUE. A WEIGHED PORTION OF THE SAMPLE IS DIGESTED WITH SULFURIC AND NITRIC ACID AT 58° C FOLLOWED BY OVERNIGHT OXIDATION WITH POTASSIUM PERMANGANATE AT ROOM TEMPERATURE. MERCURY IS SUBSEQUENTLY MEASURED BY THE CONVENTIONAL COLD VAPOR TECHNIQUE.
- 1.2 THE RANGE OF THE METHOD IS 0.2 TO 5 UG/G, BUT MAY EXTEND ABOVE OR BELOW THE NORMAL INSTRUMENT AND RECORDER CONTROL.

2. SAMPLE PREPARATION

THE SAMPLE MUST BE PREPARED BY THE SPECIAL METAL PROCEDURE CONTAINED HEREIN. A 0.2 TO 0.3G PORTION OF FROZEN SAMPLE MUST BE USED FOR EACH ANALYSIS.

3. PREPARATION OF CALIBRATION CURVE

- 3.1 THE CALIBRATION CURVE IS PREPARED FROM SPIKED TISSUE SAMPLES TREATED IN THE SAME MANNER AS THE TISSUE SAMPLES BEING ANALYZED. FOR PREPARATION OF THE CALIBRATION STANDARDS, USE A 5G PORTION OF TISSUE BLENDED IN A WARING BLENDER.
- 3.2 TRANSFER ACCURATELY WEIGHED PORTIONS TO EACH OF SIX DRY BOD BOTTLES. EACH SAMPLE MUST WEIGH APPROXIMATELY 0.2 GRAMS. ADD 4 ML OF CONC. H<sub>2</sub>SO<sub>4</sub> AND 1 ML OF CONC. HNO<sub>3</sub> TO EACH BOTTLE AND PLACE IN A WATER BATH AT 58° C UNTIL THE TISSUE IS COMPLETELY DISSOLVED (30 TO 60 MIN.).
- 3.3 COOL AND TRANSFER 0, 0.5, 1.0, 2.0, 5.0, AND 10.0 ML ALIQUOTS OF THE WORKING MERCURY SOLUTION CONTAINING 0 TO 1.0 UG OF MERCURY TO THE BOD BOTTLES. COOL TO 4° C IN AN ICE BATH AND CAUTIOUSLY ADD 15 ML OF POTASSIUM PERMANGANATE SOLUTION. ALLOW TO STAND OVERNIGHT AT ROOM TEMPERATURE UNDER OXIDIZING CONDITIONS.
- 3.4 ADD ENOUGH DISTILLED WATER TO BRING THE TOTAL VOLUME TO APPROXIMATELY 125 ML. ADD 6 ML OF SODIUM CHLORIDE-HYDROXYLAMINE SULFATE SOLUTION TO REDUCE THE EXCESS PERMANGANTE.
- 3.5 WAIT AT LEAST 30 SEC. AFTER THE ADDITION OF HYDROXYLAMINE. TREATING EACH BOTTLE INDIVIDUALLY, ADD 5 ML OF THE STANNOUS SULFATE SOLUTION AND IMMEDIATELY ATTACH THE BOTTLE TO THE AERATION APPARATUS.
- 3.6 CONTINUE WITH THE PROCEDURE AS GIVEN IN METHOD 245.1 FOR WATER (7). THE CALIBRATION CURVE IS PREPARED BY PLOTTING THE PEAK HEIGHT VERSUS THE MERCURY CONCENTRATION. THE PEAK OF THE BLANK IS SUBTRACTED FROM EACH OF THE OTHER VALUES.

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### 4. SAMPLE PROCEDURE

- 4.1 WEIGH 0.2 TO 0.3G PORTIONS OF THE SAMPLE AND PLACE IN THE BOTTOM OF A DRY BOD BOTTLE. CARE MUST BE TAKEN THAT NONE OF THE SAMPLE ADHERES TO THE SIDE OF THE BOTTLE. ADD 4 ML OF CONC. H<sub>2</sub>SO<sub>4</sub> AND 1 ML OF CONC. HNO<sub>3</sub> TO EACH BOTTLE AND PLACE IN A WATER BATH MAINTAINED AT 58° C UNTIL THE TISSUE IS COMPLETELY DISSOLVED (30 TO 60 MINUTES).
- 4.2 COOL TO 4° C IN AN ICE BATH AND CAUTIOUSLY ADD 5 ML OF POTASSIUM SOLUTION IN 1-ML INCREMENTS. ADD AN ADDITIONAL 10 ML OR MORE OF PERMANGANATE, AS NECESSARY TO MAINTAIN OXIDIZING CONDITIONS. ALLOW TO STAND OVERNIGHT AT ROOM TEMPERATURE (SEE NOTE). CONTINUE AS DESCRIBED UNDER 3.4.

NOTE: AS AN ALTERNATE TO THE OVERNIGHT DIGESTION, THE SOLUBILIZATION OF THE TISSUE MAY BE PERFORMED IN A WATER BATH AT 80° C FOR 30 MIN. THE SAMPLE MUST THEN BE COOLED AND 15 ML OF POTASSIUM PERMANGANATE SOLUTION ADDED CAUTIOUSLY. AT THIS POINT, THE SAMPLE IS RETURNED TO THE WATER BATH AND DIGESTED FOR AN ADDITIONAL 90 MIN. AT 30° C (9). IF THIS METHOD IS FOLLOWED, THE CALIBRATION STANDARDS MUST BE TREATED IN THIS MANNER. CONTINUE AS DESCRIBED UNDER 3.4.

### 5. CALCULATION

- 5.1 MEASURE THE PEAK HEIGHT OF THE UNKNOWN FROM THE CHART, AND DETERMINE MERCURY VALUE FROM THE STANDARD CURVE.
- 5.2 CALCULATE THE MERCURY CONCENTRATION IN THE SAMPLE USING THE FORMULA:

$$\text{UG HG/GRAM} = \frac{\text{UG HG IN ALIQUOT}}{\text{WT. OF ALIQUOT IN GRAMS}}$$

- 5.3 REPORT MERCURY CONCENTRATIONS AS FOLLOWS:

1. BELOW 0.1 UG/GM - REPORT AS "<0.1 UG"
2. BETWEEN 0.1 AND 1.0 UG/GM - REPORT TO NEAREST 0.01 UG
3. BETWEEN 1.0 AND 10.0 UG/GM - REPORT TO NEAREST 0.1 UG
4. ABOVE 10.0 UG/GM - REPORT TO NEAREST 1.0 UG

### 6. QUALITY ASSURANCE

- 6.1 STANDARD QUALITY ASSURANCE PROTOCOLS MUST BE EMPLOYED FOR ALL BLANKS, DUPLICATES, AND SPIKED SAMPLES AS DESCRIBED IN THE "ANALYTICAL QUALITY CONTROL HANDBOOK" (4).
- 6.2 REPORT ALL QUALITY CONTROL DATA WHEN REPORTING RESULTS OF SAMPLE ANALYSES.

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7. PRECISION AND ACCURACY

7.1 THE FOLLOWING STANDARD DEVIATIONS ON REPLICATE TISSUE SAMPLES HAVE BEEN RECORDED AT THE INDICATED LEVELS:

CONCENTRATION	STANDARD DEVIATION	COEF. OF VARIATION
0.19 UG/G	± 0.02	11.9%
0.74 UG/G	± 0.05	7.0%
2.1 UG/G	± 0.06	3.6%

RECOVERY OF MERCURY AT THESE LEVELS, ADDED AS METHYL MERCURIC CHLORIDE, WAS 112%, 93% AND 86%, RESPECTIVELY.

ANALYSIS OF TISSUE FOR METALS

1. SCOPE

THIS METHOD IS USED FOR THE DETERMINATION OF ANTIMONY, ARSENIC, BERYLLIUM, CADMIUM, CHROMIUM, COPPER, LEAD, NICKEL, SELENIUM, SILVER, THALLIUM, AND ZINC IN ANIMAL TISSUE.

2. SUMMARY OF METHOD

THE TISSUE IS PREPARED FOR ANALYSIS BY BEING CHOPPED INTO SMALL PIECES, HOMOGENIZED IN A BLENDER WITH DRY ICE, AND SOLUBILIZED BY EITHER DISSOLUTION AFTER DRY ASHING OR A WET OXIDATION DIGESTION. AFTER SAMPLE PREPARATION, ATOMIC ABSORPTION - EITHER DIRECT ASPIRATION, GASEOUS HYDRIDE, OR A FLAMELESS TECHNIQUE - IS USED TO MEASURE THE CONCENTRATION OF THE POLLUTANT.

3. PRESERVATION AND HANDLING

ALTHOUGH AN ALIQUOT OF THE GROUND TISSUE MAY BE USED FOR THE METALS DETERMINATION, IT MAY BE MORE DESIRABLE TO PREPARE AN INDIVIDUAL SAMPLE TO AVOID POSSIBLE METAL CONTAMINATION FROM THE GRINDER. DUST IN THE LABORATORY ENVIRONMENT, IMPURITIES IN REAGENTS, AND IMPURITIES ON LABORATORY APPARATUSES ARE ALL SOURCES OF POTENTIAL CONTAMINATION. ALL GLASSWARE MUST BE THOROUGHLY WASHED WITH DETERGENT AND TAP WATER, THEN RINSED WITH A 1:1 NITRIC ACID SOLUTION (ONE PART NITRIC ACID TO ONE PART TAP WATER), AND GIVEN A FINAL RINSE WITH DEIONIZED, DISTILLED WATER.

NOTE: CHROMIC ACID MAY BE USEFUL TO REMOVE ORGANIC DEPOSITS FROM GLASSWARE; HOWEVER, THE ANALYST MUST BE CAUTIONED THAT THE GLASSWARE MUST BE THOROUGHLY RINSED WITH WATER TO REMOVE THE LAST TRACE OF CHROMIUM. THIS IS ESPECIALLY IMPORTANT IF CHROMIUM IS TO BE INCLUDED IN THE ANALYTICAL SCHEME. A COMMERCIAL PRODUCT - NOCHROMIX - AVAILABLE FROM GODAX LABORATORIES, 6 VARICK STREET, NEW YORK, NY, 10013, MAY BE USED IN PLACE OF CHROMIC ACID.

4. SAMPLE HOMOGENIZATION

4.1 UNWRAP AND WEIGH THE FROZEN TISSUE.

4.2 THE TISSUE MUST BE CHOPPED INTO APPROXIMATELY 1-IN OR SMALLER CHUNKS WITH A MEAT CLEAVER OR A KNIFE AND Mallet (2 TO 3-LB). SMALLER PIECES ENSURE EFFICIENT GRINDING.

4.3 PLACE CRUSHED OR PELLETED DRY ICE INTO THE BLENDER CONTAINER. THE WEIGHT OF DRY ICE SHOULD BE EQUAL TO, OR GREATER THAN, THE WEIGHT OF THE TISSUE.

4.4 TURN ON THE BLENDER FOR 10 SEC. TO PULVERIZE THE ICE AND CHILL THE BLENDER.

4.5 ADD THE PIECES OF TISSUE AND BLEND AT HIGH SPEED UNTIL THE MIXTURE IS HOMOGENEOUS. THIS USUALLY REQUIRES 2 TO 5 MINUTES. ADD MORE DRY ICE IF NEEDED TO KEEP THE TISSUE FROZEN.

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- 4.6 POUR THE HOMOGENATE INTO A PLASTIC BAG AND CLOSE THE BAG WITH A RUBBER BAND. DO NOT SEAL THE BAG TIGHTLY TO ALLOW CO<sub>2</sub> TO ESCAPE.
- 4.7 PLACE THE BAG IN THE FREEZER (-12°C FOR AT LEAST 16 HRS) UNTIL READY TO PROCEED WITH THE DIGESTION STEP.

NOTE: IF DESIRED, THE BLENDER BLADES CAN BE MODIFIED IN ORDER TO HAVE THE LEADING EDGE OF THE BLADES (THE SHARPENED EDGE) TURNED DOWN SO THAT, AS IT ROTATES, THE BLADE WILL THROW THE MATERIAL UPWARDS. BECAUSE STAINLESS STEEL BLADES MAY BE A POSSIBLE SOURCE OF NICKEL AND CHROMIUM CONTAMINATION, A TANTALUM BLADE SHOULD BE SUBSTITUTED FOR THE STAINLESS STEEL IF AVAILABLE.

THE HOLE IN THE BLENDER LID SHOULD BE ENLARGED SUFFICIENTLY TO ALLOW THE EVOLVED GAS TO ESCAPE. HOLD A CLOTH OR LABWIPE OVER THIS HOLE WHEN BLENDING TO PREVENT LOSS OF THE SAMPLE MATERIAL. A GLOVE MUST BE WORN TO PREVENT POSSIBLE FREEZING OF THE SKIN BY ESCAPING GAS.

## 5. REAGENTS

- 5.1 DEIONIZED, DISTILLED WATER: PREPARE BY PASSING DISTILLED WATER THROUGH A MIXED BED OF CATION AND ANION EXCHANGE RESINS. USE DEIONIZED, DISTILLED WATER FOR THE PREPARATION OF ALL REAGENTS, CALIBRATION STANDARDS AND DILUTION WATER.
- 5.2 NITRIC ACID (CONC.): IF METAL IMPURITIES ARE PRESENT, DISTILL REAGENT GRADE NITRIC ACID IN A BOROSILICATE GLASS DISTILLATION APPARATUS.
- 5.3 SULFURIC ACID, ACS GRADE (95.5% TO 96.5%).
- 5.4 SULFURIC ACID - 20% V/V SOLUTION. CAREFULLY ADD 200 ML OF CONCENTRATED H<sub>2</sub>SO<sub>4</sub> TO 500 ML OF WATER. COOL AND DILUTE TO 1 LITER WITH WATER.
- 5.5 HYDROCHLORIC ACID, ACS GRADE.
- 5.6 HYDROGEN PEROXIDE, 50% STABILIZED ACS GRADE.
- 5.7 DRY ICE (FROZEN CARBON DIOXIDE), PELLET FORM PREFERRED.

## 6. APPARATUS

- 6.1 BLENDER, WARING, TWO-SPEED; STAINLESS STEEL OR TANTALUM BLADE, IF AVAILABLE; GLASS CONTAINER CAPACITY 1000 ML OR EQUIVALENT EQUIPMENT.
- 6.2 DRYING OVEN - CONTROLLABLE WITH THE RANGE OF 100° to 150°C WITH LESS THAN ± 5°C VARIATION. CHECK CALIBRATION OF OVEN TEMPERATURE CONTROL TO ENSURE ACCURATE ASHING TEMPERATURES. FURNACE MUST BE OPERATED IN SUITABLE FUME HOOD.
- 6.3 HOT PLATE, CONTROLLABLE WITHIN THE RANGE OF 80° TO 400° C. HOT PLATE MUST BE OPERATED IN FUME HOOD.



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7. PROCEDURE

EXCEPT FOR MERCURY WHICH REQUIRES A COLD VAPOR TECHNIQUE, THE METALS CAN BE DIVIDED INTO TWO GROUPS FOR CONTINUED PROCESSING.

GROUP I: Be, Cd, Cr, Cu, Pb, Ni, Ag, Tl, AND Zn.

GROUP II: As AND Se.

GROUP I IS DIGESTED BY A DRY ASHING PROCESS (10) WITH THE USE OF AN ASHING AID; GROUP II IS PREPARED UTILIZING A WET ASHING PROCESS.

7.1 GROUP I - METALS

7.1.1 REMOVE THE HOMOGENIZED SAMPLE FROM FREEZER AND WEIGH APPROXIMATELY 10G INTO A TARED, 100-ML TALL FORM, PYREX BEAKER. SUBTRACT THE BEAKER WEIGHT FROM THE TOTAL AND RECORD THE WET SAMPLE WEIGHT.

7.1.2 ADD 25 ML OF 20% SULFURIC ACID. MIX EACH SAMPLE THOROUGHLY WITH A GLASS STIRRING ROD TO ENSURE THAT ALL SAMPLE MATERIAL IS WETTED BY THE ACID. RINSE THE STIRRING ROD WITH WATER INTO THE ASHING VESSEL AND COVER THE SAMPLE WITH A RIBBED WATCH GLASS.

7.1.3 HEAT THE SAMPLES IN AN OVEN OR FURNACE AT  $110 \pm 5^{\circ}\text{C}$  UNTIL A CHARRED VISCOUS SULFURIC ACID/SAMPLE RESIDUE REMAINS; 12 TO 16 HRS. (OVERNIGHT) IS USUALLY SUFFICIENT. TRANSFER THE ASHING VESSELS CONTAINING THE SAMPLES TO A COLD, CLEAN MUFFLE FURNACE WITH GOOD EXTERNAL VENTILATION (FUME HOOD), MAKING SURE THAT THE SAMPLE REMAINS COVERED DURING THE TRANSFER. INITIALLY THE FURNACE SHOULD BE SET AT  $125^{\circ}\text{C}$ ; THE TEMPERATURE SHOULD BE INCREASED APPROXIMATELY EVERY HOUR IN  $50^{\circ}$  INCREMENTS TO  $275^{\circ}\text{C}$ . MAINTAIN THIS TEMPERATURE FOR 3 HOURS, INCREASE THE TEMPERATURE TO  $450^{\circ}\text{C}$  (AT  $50^{\circ}$  PER HOUR), AND MAINTAIN THIS TEMPERATURE 16 HRS (OVERNIGHT). REMOVE THE COVERED ASHING VESSELS FROM THE FURNACE AND ALLOW TO COOL TO ROOM TEMPERATURE IN A CLEAN, DRAFT-FREE AREA.

7.1.4 AFTER INITIAL OVERNIGHT ASHING, SOME RESIDUAL CARBON MAY REMAIN IN THE SAMPLES. TREAT EACH SAMPLE ASH WITH 0.5 ML OF WATER AND 1 ML OF CONCENTRATED NITRIC ACID (WHETHER OR NOT THEY ARE ALREADY WHITE). EVAPORATE CAREFULLY JUST TO DRYNESS ON A WARM HOTPLATE (IN A FUME HOOD). PLACE THE ASHING VESSELS (COVERED WITH WATCH GLASSES) IN A COOL MUFFLE FURNACE AND RAISE THE TEMPERATURE TO  $300^{\circ}\text{C}$ ; MAINTAIN THIS TEMPERATURE FOR EXACTLY 30 MIN. REMOVE EACH COVERED SAMPLE ASH FROM THE FURNACE AND ALLOW TO COOL AS BEFORE. IF RESIDUAL CARBON REMAINS, REPEAT THE NITRIC ACID TREATMENT UNTIL A CARBON-FREE WHITE ASH IS OBTAINED. THE COVERED ASHING VESSELS CONTAINING THE ASH MUST BE STORED IN A DESSICATOR OR IN A LAMINAR FLOW CLEAN HOOD.

NOTE: COPIOUS CARBON RESIDUES (i.e., BLACK ASHES) AFTER OVERNIGHT ASHING ARE INDICATIVE OF INEFFICIENT OR UNEVEN HEATING WITHIN THE FURNACE. ROUTINE CALIBRATION OF THE FURNACE IS REQUIRED.

7.1.5 ADD 0.5 ML OF NITRIC ACID AND 10 ML OF WATER TO EACH COOL ASHING VESSEL AND WARM GENTLY ON A HOTPLATE AT  $80^{\circ}$  TO  $90^{\circ}$  FOR 5 TO 10 MIN.

TO EFFECT DISSOLUTION OF THE ASH. A SMALL AMOUNT OF INSOLUBLE WHITE SILICEOUS-LIKE RESIDUE MAY REMAIN UNDISSOLVED; DO NOT FILTER THE RESIDUE AS THIS WILL INCREASE THE POSSIBILITY OF CONTAMINATION. QUANTITATIVELY TRANSFER THE CONTENTS OF EACH ASHING VESSEL INTO A 100 ML VOLUMETRIC FLASK, DILUTE TO VOLUME WITH WATER, AND SHAKE THOROUGHLY. ALLOW ANY RESIDUE TO SETTLE TO THE BOTTOM OF THE FLASK (ABOUT 2 HR). DO NOT SHAKE THE SAMPLE FURTHER BEFORE TAKING AN ALIQUOT. THE SAMPLE IS NOW READY FOR ANALYSIS.

NOTE: THE PRESENCE OF A PRECIPITATE OTHER THAN THE INSOLUBLE SILICEOUS-LIKE MATERIAL MAY RESULT IN LOW OR ERRATIC RESULTS FOR Pb. PRECIPITATE FORMATION CAN RESULT FROM HEATING THE SAMPLES TOO LONG OR AT TOO HIGH A TEMPERATURE AFTER NITRIC ACID TREATMENT OF THE ASH. PRECIPITATE FORMATION MUST BE AVOIDED BY MAINTENANCE OF APPROPRIATE ASHING TEMPERATURES.

- 7.1.6 THE PREPARED SAMPLE MUST BE ANALYZED BY ATOMIC ABSORPTION. FOR A DISCUSSION OF BASIC PRINCIPLES, THE METHOD OF STANDARD ADDITION, THE CHELATION/SOLVENT EXTRACTION PROCEDURES, GENERAL INSTRUMENTAL OPERATING PARAMETERS, AND PREPARATION OF STANDARDS AND CALIBRATION, SEE THE SECTION ON "ATOMIC ABSORPTION METHODS" (7), AND THE INDIVIDUAL ANALYSES SHEETS AS FOLLOWS:

ELEMENT	Ag	Be	Cd	Cr	Cu	Ni	Pb	Se	Tl	Zn
METHOD	272.1	210.1	213.1	218.1	220.1	249.1	239.1	204.1	279.1	289.1

- 7.1.7 BECAUSE OF THE ADEQUATE SENSITIVITY BY CONVENTIONAL FLAME AA AND THE EXPECTED CONCENTRATION LEVELS OF CADMIUM, COPPER, AND ZINC IN THE SAMPLE, THESE THREE ELEMENTS MUST BE ANALYZED VIA DIRECT ASPIRATION. BECAUSE OF THEIR EXPECTED LOW CONCENTRATIONS, THE FURNACE TECHNIQUE IS PREFERRED FOR THE ANALYSIS OF THE OTHER GROUP I METALS. WHEN USING THE FURNACE TECHNIQUE, THE OPERATING PARAMETERS AND INSTRUCTIONS AS SPECIFIED BY THE PARTICULAR INSTRUMENT MANUFACTURER MUST BE FOLLOWED. IF THE CONCENTRATION DETECTED BY THE FURNACE PROCEDURE IS BEYOND THE WORKING RANGE OF THE STANDARD CURVE, THE SAMPLE MUST EITHER BE DILUTED AND REANALYZED OR ANALYZED VIA DIRECT ASPIRATION. THE METHOD OF STANDARD ADDITIONS MUST BE EMPLOYED WHEN NEEDED. IF THE SAMPLE MATRIX IS SO COMPLEX THAT SAMPLE DILUTION FOLLOWED BY FURNACE ANALYSIS CANNOT BE USED, OR IF THE USE OF THE CHELATION/SOLVENT EXTRACTION TECHNIQUE FOR CONCENTRATIONS OF Ag, Ni, Pb, AND Tl IS PREFERRED, THE PROCEDURE DESCRIBED IN METHODS FOR CHEMICAL ANALYSIS OF WATER AND WASTES, REFERENCE 7, SHOULD BE UTILIZED.

## 7.2 GROUP II - METALS

- 7.2.1 REMOVE THE HOMOGENIZED SAMPLE FROM THE FREEZER AND WEIGH APPROXIMATELY 5G INTO A TARED, 120-ML CONICAL BEAKER. SUBTRACT THE BEAKER WEIGHT FROM THE TOTAL AND RECORD THE WET SAMPLE WEIGHT.
- 7.2.2 ADD 5 ML OF CONC. HNO<sub>3</sub>. SLOWLY ADD 6 ML OF CONC. H<sub>2</sub>SO<sub>4</sub> AND COVER WITH A WATCH GLASS.
- 7.2.3 PLACE THE BEAKER ON A HOT PLATE AND WARM SLIGHTLY. CONTINUE HEATING UNTIL THE MIXTURE BECOMES DARK OR A POSSIBLE REDUCING

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CONDITION IS EVIDENT. DO NOT ALLOW THE MIXTURE TO CHAR. REMOVE THE BEAKER FROM THE HOTPLATE AND ALLOW TO COOL.

NOTE: REMOVE BEAKER IF FOAMING BECOMES EXCESSIVE.

- 7.2.4 ADD AN ADDITIONAL 5 ML OF CONC.  $\text{HNO}_3$ , COVER WITH A WATCH GLASS, AND RETURN THE BEAKER TO THE HOTPLATE. REPEAT STEP 7.2.3.
- 7.2.5 WHEN THE MIXTURE AGAIN TURNS BROWN, COOL, AND SLOWLY ADD 5 ML OF 50% HYDROGEN PEROXIDE. COVER WITH A WATCH GLASS AND HEAT GENTLY UNTIL THE INITIAL REACTION HAS CEASED. IF THE SOLUTION BECOMES DARK, REPEAT THE PEROXIDE ADDITION, SEVERAL TIMES IF NECESSARY, AND HEAT SO THAT  $\text{SO}_2$  FUMES APPEAR. IF CHARRING OCCURS, ADD ADDITIONAL 1 ML PORTIONS OF HYDROGEN PEROXIDE UNTIL THE FUMING SULFURIC ACID REMAINS COLORLESS OR VERY LIGHT YELLOW. (IF AT ANY STAGE IT APPEARS THAT THE SULFURIC ACID MAY APPROACH DRYNESS, COOL, ADD 2 TO 3 ML OF SULFURIC ACID, AND CONTINUE.)
- 7.2.6 COOL, ADD 40 ML OF CONC.  $\text{HCl}$  AND DILUTE TO 100 ML WITH DEIONIZED, DISTILLED WATER. THE SAMPLE IS NOW READY FOR ANALYSIS.
- 7.2.7 THE GROUP II METALS MUST BE ANALYZED VIA ATOMIC ABSORPTION USING THE GASEOUS HYDRIDE TECHNIQUE. THE APPARATUS SETUP, STANDARD PREPARATION AND CALIBRATION, AND ANALYSIS PROCEDURE TO BE FOLLOWED IS PRESENTED STARTING ON PAGE 159, REFERENCE 8. FROM THE PREPARED SAMPLE, A 25-ML ALIQUOT SHOULD BE WITHDRAWN AND THE ANALYSIS CONTINUED AS DESCRIBED IN SECTION 3.d, PAGE 162, REFERENCE 8.

8. CALCULATION

USING THE VALUES FROM THE APPROPRIATE CALIBRATION CURVE, CALCULATE THE CONCENTRATION OF EACH METAL POLLUTANT IN THE TISSUE AS FOLLOWS:

IF THE CONCENTRATION OF STANDARDS IN THE CALIBRATION CURVE IS PLOTTED AS  $\text{MG/L}$ ,

$$\text{UG/GRAM} = \frac{\begin{array}{l} \text{MG/L OF CONSTITUENT} \\ \text{IN PREPARED SAMPLE} \end{array} \times \begin{array}{l} \text{VOLUME OF PREPARED} \\ \text{SAMPLE IN ML} \end{array}}{\text{WEIGHT OF WET SAMPLE IN GRAMS}}$$

IF THE CONCENTRATION OF STANDARDS IN THE CALIBRATION CURVE IS PLOTTED AS  $\text{UG/L}$ ,

$$\text{UG/GRAM} = \frac{\begin{array}{l} \text{UG/L OF CONSTITUENT} \\ \text{IN PREPARED SAMPLE} \end{array} \times \begin{array}{l} \text{VOLUME OF PREPARED} \\ \text{SAMPLE IN LITERS} \end{array}}{\text{WEIGHT OF WET SAMPLE IN GRAMS}}$$

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9. QUALITY ASSURANCE

- 9.1 STANDARD QUALITY ASSURANCE PROTOCOLS MUST BE EMPLOYED, INCLUDING BLANKS, DUPLICATES, AND SPIKED SAMPLES AS DESCRIBED IN THE "ANALYTICAL QUALITY CONTROL HANDBOOK" (4).
- 9.2 REPORT ALL QUALITY CONTROL DATA WHEN REPORTING RESULTS OF SAMPLE ANALYSES.

## REFERENCES

1. "METHODS FOR BENZIDINE, CHLORINATED ORGANIC COMPOUNDS, PENTACHLOROPHENOL AND PESTICIDES IN WATER AND WASTEWATER," U.S. ENVIRONMENTAL PROTECTION AGENCY, ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY, CINCINNATI, OHIO, 45268, 1978.
2. FEDERAL REGISTER, VOLUME 41, NO. 232, P. 52780, WEDNESDAY, DECEMBER 1976.
3. FEDERAL REGISTER, VOLUME 44, NO. 233, P. 69464, MONDAY, DECEMBER 3, 1979.
4. HANDBOOK FOR ANALYTICAL QUALITY CONTROL IN WATER AND WASTEWATER LABORATORIES. U.S. ENVIRONMENTAL PROTECTION AGENCY, TECHNOLOGY TRANSFER, 1979.
5. BOYLE, H. W., ET AL., ADV. CHEM. SERV., 60, 207 (1966).
6. STALLING, D. L.; R. C. TINDLE; J. L. JOHNSON; "CLEANUP OF PESTICIDE AND POLYCHLORINATED BIPHENYL RESIDUES IN FISH EXTRACTS BY GEL PERMEATION CHROMATOGRAPHY." JOAC, 55, 32-38 (1972).
7. "METHODS FOR CHEMICAL ANALYSIS OF WATER AND WASTES," U.S. ENVIRONMENTAL PROTECTION AGENCY, TECHNOLOGY TRANSFER. (1979).
8. "STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER," 14TH EDITION (1975).
9. BISHOP, J.N., "MERCURY IN FISH," ONTARIO WATER RESOURCES COMM., TORONTO, ONTARIO, CANADA, 1971.
10. JONES, J. W.; R. J. GAJAN; K. W. BOYER; J. A. FIORINO; "DRY ASH - VOLTAMMETRIC DETERMINATION OF CADMIUM, COPPER, LEAD, AND ZINC IN FOODS." JOAC, 60, 826. (1977).

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B7

B183



## United States Department of the Interior

FISH AND WILDLIFE SERVICE  
Great Lakes Fishery Laboratory  
1451 Green Road  
Ann Arbor, Michigan 48105

IN REPLY REFER TO:

March 1, 1985

Dr. Thomas Dillon  
Environmental Laboratory  
Waterways Experiment Station  
U.S. Army Corps of Engineers  
P.O. Box 631  
Vicksburg, Mississippi 39180

Dear Tom:

No doubt the evaluation protocol for open water disposal of sediments into fresh water will involve a series of tests. The "Flow Chart of Dredging Project Evaluation" (enclosed) proposed by the Dredging Subcommittee of the International Joint Commission lists three types of tests under sediment bioassessment; acute toxicity, bioaccumulation, and reproductive impairment. In light of these guidelines and the recommendations that we presented to the US EPA in a literature review (EPA-905/3-84-005), our laboratory has been conducting research on a bioaccumulation test. Our bioaccumulation test was developed from the one used in the ocean discharge criteria and consists of a 10-day, flow-through exposure to whole sediment. In our testing we have been able to demonstrate several aspects of the test that substantiate its utility:

- (a) 10 days is adequate for determination of bioaccumulation potential from sediment although maximum or steady-state concentrations will not be attained.
- (b) A benthic invertebrate and fish species should be included in the test. Good results have been obtained using the common nightcrawler (Lumbricus terrestris) and fathead minnow (Pimephales promelas). Both species are relatively tolerant of a wide range of conditions, are efficient accumulators and easily provide ample tissue for analysis.
- (c) Organisms must be allowed physical contact with sediments. This more closely simulates field conditions for bioaccumulation and eliminates the need to mechanically resuspend sediment.
- (d) Low flow rates will keep experimental conditions constant without washing out fine materials. We've had good success with a flow rate of 0.1 L/min in 39L aquaria.

If you have any further questions please don't hesitate to call (FTS 378-1245). I look forward to participating in the workshop.

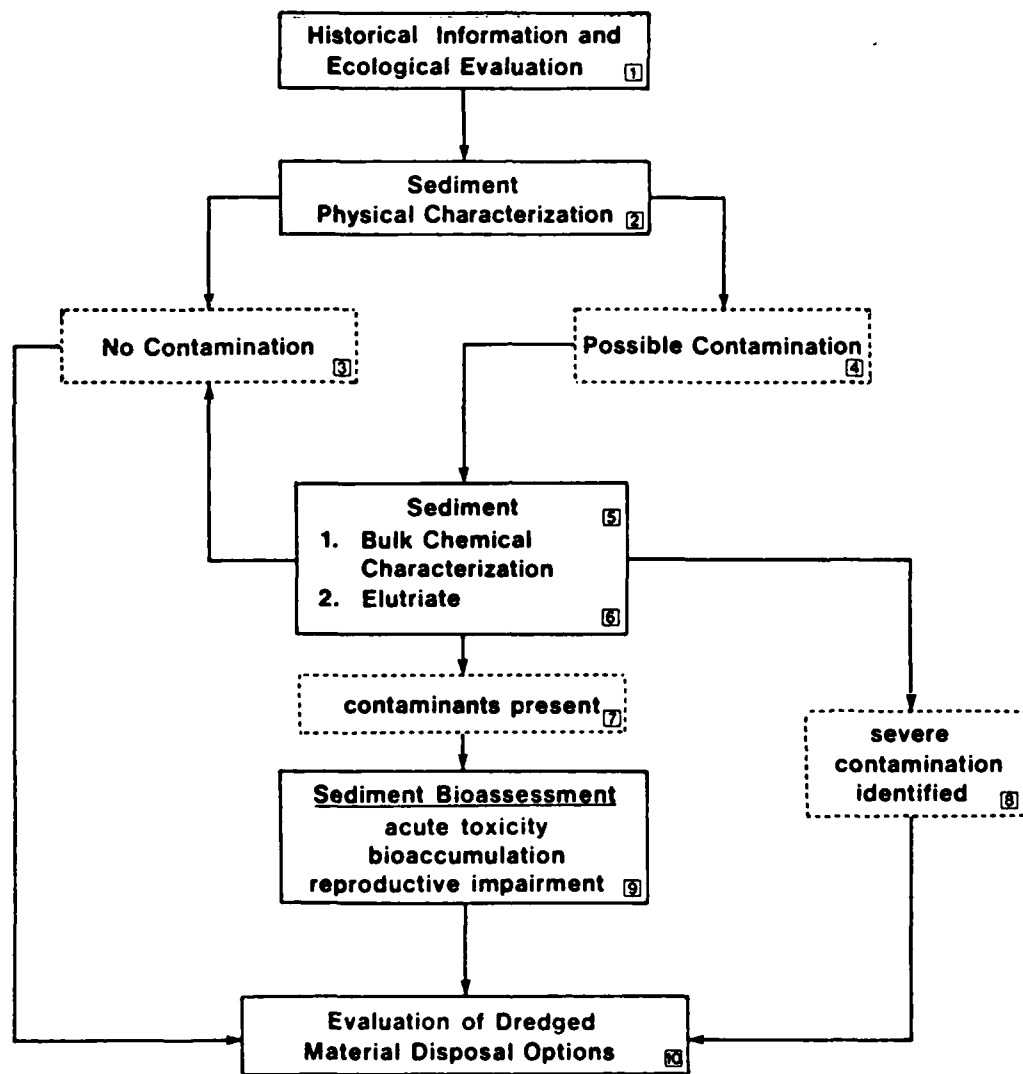
Sincerely,

A handwritten signature in cursive script, appearing to read "Mike".

Michael J. Mac



Fig. 4 FLOW CHART OF DREDGING PROJECT EVALUATION



United States  
Environmental Protection  
Agency

Great Lakes National  
Program Office  
536 South Clark Street  
Chicago, Illinois 60605

EPA 905 3 84 007  
November 1984



# Flow-through Bioassay For Measuring Bioaccumulation of Toxic Substances From Sediment



**Flow-through Bioassay For Measuring  
Bioaccumulation of Toxic Substances From Sediment**

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**U.S. Fish and Wildlife Service  
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**Final Report  
May 1984  
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**1/Contribution No. 616 of the Great Lakes Fishery Laboratory**

DISCLAIMER

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## FOREWORD

The Great Lakes National Program Office (GLNPO) of the United States Environmental Protection Agency was established in Region V, Chicago, to focus attention on the significant and complex natural resource represented by the Great Lakes.

GLNPO implements a multi-media environmental management program drawing on a wide range of expertise represented by universities, private firms, State, Federal, and Canadian governmental agencies, and the International Joint Commission. The goal of the GLNPO program is to develop programs, practices and technology necessary for a better understanding of the Great Lakes Basin ecosystem and to eliminate or reduce to the maximum extent practicable the discharge of pollutants into the Great Lakes system. GLNPO also coordinates U.S. actions in fulfillment of the Great Lakes Water Quality Agreement of 1978 between Canada and the United States of America.

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## Introduction

Over 10 million cubic meters of sediment are dredged annually from Great Lakes waterways. Because much of this material is taken from harbors, connecting channels, and other nearshore areas that often are contaminated with toxic substances, the sediments proposed for dredging need to be evaluated for the presence of bioavailable contaminants and the potential for toxicity to the biota. Sound decisions on the appropriate disposal of the dredged material can be made only after such an evaluation. Presently, no standardized procedure exists for evaluating dredged material in freshwater systems although criteria for discharge of dredged material into marine waters have been developed (USEPA/CE 1977). In the ocean discharge guidelines, it is recommended that bioassays be conducted on liquid, solid, and suspended particulate phases of dredged material. Because it appears that the solid phase has the greatest potential for environmental damage and because measurements of bioaccumulation must be made to evaluate sediments for disposal (USEPA/CE 1977, Seelye and Mac 1983), we developed a bioassay for testing the solid phase of dredged material that measures the survival of organisms and, perhaps more important, the bioaccumulation of toxic substances. Although other workers have demonstrated the bioaccumulation of toxic substances by aquatic organisms from naturally contaminated sediments (Peddicord et al. 1980; Rubinstein et al. 1980, 1983; Seelye et al. 1982), several have used testing methods that result in unacceptable mortality to control organisms (Bahnick et al. 1981, Prater et al. 1983).

Our bioassay is intended to estimate the potential for bioaccumulation of contaminants from sediments that are not acutely toxic to test organisms, but

are suspected of containing persistent contaminants. By using test organisms that are not highly susceptible to toxic compounds, the bioaccumulation test allows estimation of the potential food-chain accumulation of contaminants that may occur in local biota from surficial sediments. In practice, bioaccumulation observed in this bioassay by organisms exposed to test sediments (sediments to be dredged) would be compared to bioaccumulation observed from sediments collected from a reference site (e.g. a disposal site or open lake), and also from control sediments (relatively clean sediment). Decisions could then be based on a comparison of results between test and reference sediments to determine if disposal would cause degradation to the habitat, and between reference and control sediment to determine if even the reference material is seriously contaminated. Although the test is not intended to be a toxicity test per se, use of test, reference, and control sediments enables interpretation of any mortality of organisms that may occur during the bioassays. High mortality in bioassays with test or reference sediment would indicate acute toxicity of sediments in the project area. However if high mortality occurs in all three sediments, it can be assumed that the organisms were not in a healthy state at the time of testing.

We describe the results of 10-day sediment bioassays in which both mortality and bioaccumulation were measured in four aquatic organisms. We exposed two infaunal organisms and two species of fish to test and control sediments in the laboratory.

#### Materials and Methods

Sediment bioassays were conducted in a flow-through system consisting of eight 39-L glass tanks (Fig. 1). Each tank received 100 mL/min of 20°C water,

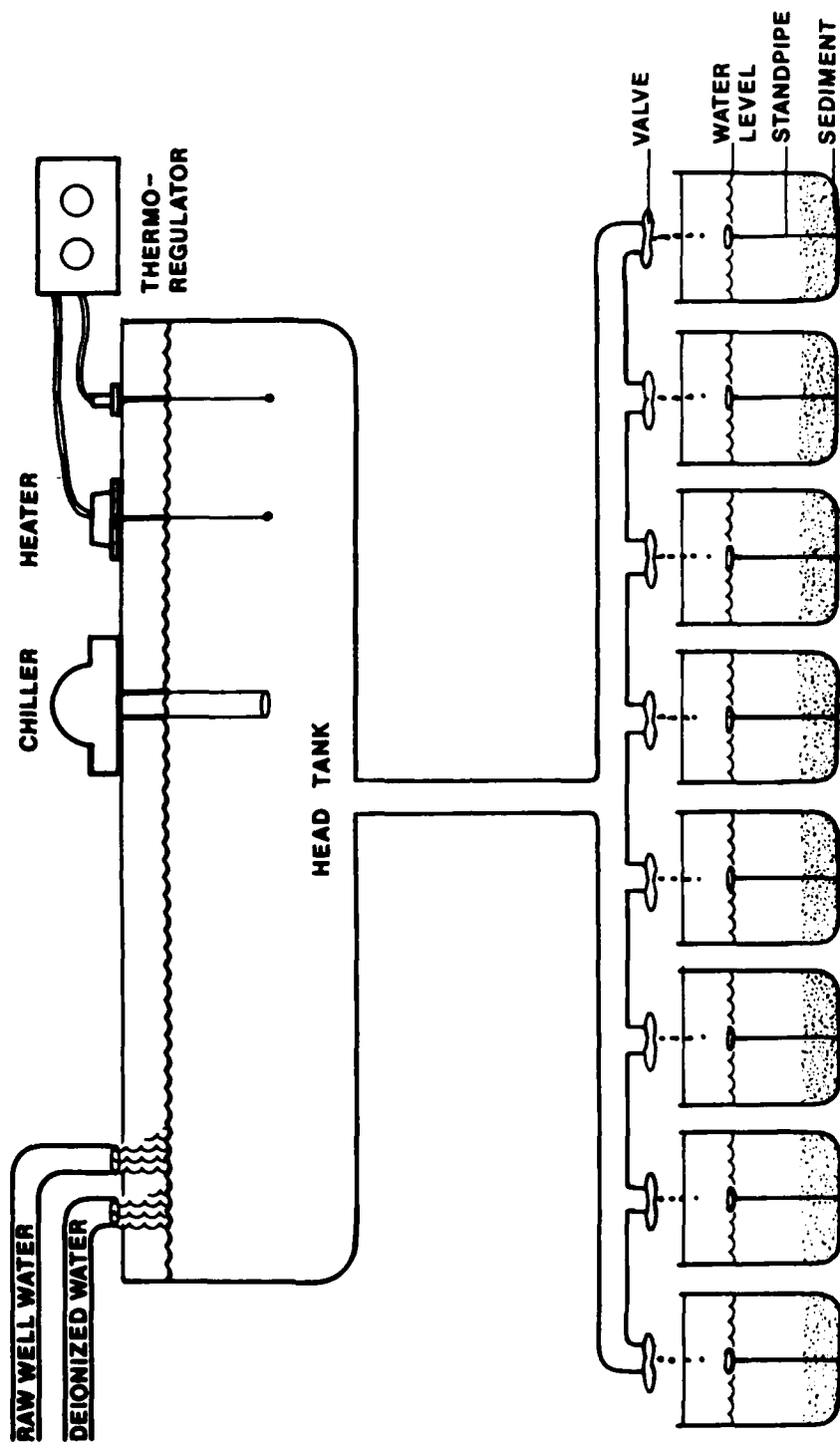


Figure 1. Diagram of sediment exposure system.

softened to a hardness of about 120 mg/L (as CaCO<sub>3</sub>) by mixing deionized well water with processed well water (hardness 442 mg/L, Seelye et al. 1982). Prior to the start of a test, about 11 kg (5 cm depth) of sediment was added to each tank; four tanks received contaminated (test) sediment and four received clean (control) sediment. Water was then added to the tank and the sediment was allowed to settle for 24 hours before test organisms were added. Tests lasted 12 days. Organisms were exposed to sediment for 10 days and then moved to identical tanks containing only flowing water for 2 days to allow for clearance of ingested sediment from the gut. During the tests we monitored water temperature, flow rate, hardness, dissolved oxygen, and sediment redox potential. Suspended solids were measured only in the exposures involving fish.

We tested two species of fish (fathead minnows, Pimephales promelas, and yellow perch, Perca flavescens) and two species of invertebrates (an oligochaete worm, Octolasion tyrtaeum, and the Asiatic clam, Corbicula fluminea). Adult minnows (2-3g) and juvenile perch (2g) were obtained from the National Fishery Research Laboratory, La Crosse, Wisconsin; the worms were collected from the Black River near Onaway, Michigan; and the clams were obtained from the Sacramento River delta in California. Test organisms were held at the Great Lakes Fishery Laboratory for at least 2 weeks and acclimated to softened water for at least 5 days before testing. During this time, fish were fed Silver Cup<sup>1/</sup> pellets, clams were fed algae (Chlorella), and oligochaetes were maintained in forest duff in which organic matter was available for food. Organisms were not fed during the tests.

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<sup>1/</sup>Reference to trade names does not imply U.S. Government endorsement

During testing, we also examined the suitability of two other invertebrates: Chironomus larvae and Hexagenia limbata. Chironomus larvae are easily cultured and an important food chain member in the Great Lakes. We rejected them as a test organism, however, because their small size would require large numbers of similarly aged individuals to obtain enough tissue for contaminant analysis. Hexagenia limbata is another important food item in the Great Lakes. We observed high mortality during both holding and testing of this species. High mortality of H. limbata has occurred in other published sediment studies (Bahnick et al. 1981). Because of the small size of Chironomus and the high mortality of H. limbata, no further tests of these organisms were attempted.

Either 10 fish, 14 oligochaete worms, or 30 clams were placed into each tank at the start of the bioassay. We netted fish and collected worms and clams by hand following 10 days of exposure to sediments. At the end of a test, we froze all live organisms whole for contaminant analysis. In addition, a sample of test organisms was frozen prior to the start of the test for determination of preexposure contaminant concentrations. All organisms were thawed and the clams shelled before homogenization and analysis.

Control and test sediments were collected just before each test to minimize chemical changes in the sediment caused by storage (Table 1). Three sets of test sediments were collected with a ponar dredge from the Raisin River near Monroe, Michigan (41° 54' 1" N, 83° 21' 18" W), and three sets of control sediments were shoveled from Meadowood Pond in Saline Township, Michigan (42° 7' 44" N, 83° 47' 45" W). In the first exposure, two sets of tanks were used with fathead minnows in one set and oligochaetes in the other. Yellow perch were tested in sediments from the second collection and clams

Table 1. Physical and chemical characteristics of sediments from the three collections. Dates of collection and dates of various tests are also presented.

Exposures and dates of test	Date Collected	Physical composition (% dry wt.)				Contaminants (ug/g dry wt.)	
		Sand	Silt	Clay	Volatile solids	PCBs	Zn
1. Fathead minnows and oligochaetes (10/20-11/2) Control Test	10/12	65	19	13	2	0.016	34
	9/30	11	43	35	8	31.72	244
2. Yellow perch (11/16-11/28) Control Test	11/9	51	24	22	3	0.013	31
	10/25	67	16	11	4	12.78	147
3. Asiatic clams (1/28-2/9) Control Test	1/13	16	41	32	10	0.014	67
	1/14	36	33	22	7	19.60	162

were tested in sediments from the third collection. Subsamples of sediment were taken before placement in test tanks and frozen for later chemical and physical analysis.

Sediments and test organisms were analyzed for PCBs and Zn to examine the bioaccumulation potential of both an organic and an inorganic contaminant directly from contaminated sediment. We analyzed PCBs in sediment and in test organisms by gas chromatography (GC), using methods previously described by Seelye et al. (1982). Samples of tissue and sediment for Zn analysis were digested in HNO<sub>3</sub> according to the method of May and Brumbaugh (1982) except that no perchloric acid was used, and Zn concentrations were determined on a Perkin Elmer Model 228 atomic absorption spectrophotometer equipped with an HGA2200 graphite furnace. Absorption values for 50- $\mu$ L samples were compared at 307.6 nm with known standards. Graphite furnace conditions were as follows: nitrogen flow, 20 mL/min; sample drying time, 60 sec with a 15-sec ramp (20-120°C); ashing time, 50 sec with a 10-sec ramp (120° -500°C); and atomization, 6 seconds at 2200°C.

We report concentrations of PCBs and Zn in both sediment and tissue on a dry weight basis to alleviate discrepancies caused by varying water content. Thus subsamples of all analytical samples were dried to measure water content. A wet-to-dry conversion factor was calculated and applied to measured wet-weight concentrations.

We compared PCB and Zn concentrations in test organisms between preexposure samples and test and control samples after exposure, using analysis of variance (ANOVA). For both species of fish and the oligochaetes, all surviving test organisms in a tank were composited to form one analytical

sample. Thus different tanks were considered replicates. Clams remaining alive at the completion of the bioassay were divided into two analytical samples so that within- and between-tank replicates, as well as replicate tanks, were considered in the ANOVA.

### Results

The two sediments used provided contrasting levels of PCBs and Zn. Concentrations in test sediment (micrograms per gram, dry weight) ranged from 12.8 to 31.7 PCB and from 147 to 241 for Zn whereas those in control sediment were  $< 0.02$  for PCB and from 31 to 67 for Zn (Table 1). Although sediment was collected three times, which resulted in some variation in both physical and chemical composition, a large difference in the two contaminants of interest between control and test sediment was always found.

Use of the flow-through bioassay produced nearly constant conditions throughout testing, and mortality was low ( $\leq 8.3\%$ ) in test organisms, indicating that the test sediments were not acutely toxic to the organisms tested (Table 2). Mortality was high in only one test where a mechanical failure restricted flow to two tanks holding oligochaetes during exposure. In these two tanks, all oligochaetes died and were not analyzed. Thus in the contaminated sediment treatment only two replicates are reported.

All test organisms accumulated significant ( $P \leq 0.05$ ) amounts of PCBs from test sediments when compared with either organisms exposed to control sediments or preexposure organisms (Table 3). Bioaccumulation factors (BF = dry wt concentration in tissue divided by dry wt concentration in sediment) for organisms exposed to test sediments indicated that oligochaetes were the most



Table 2. Summary of test conditions and mortality of organisms.

Organism and exposure (No. of organisms in parentheses)	Test Conditions			Sediment Eh (mV)	Mortality (%)
	Temp. (°C)	Dissolved oxygen (mg/L)	Hardness (mg/L, CaCO <sub>3</sub> )		
<b>Fathead minnows</b>					
Control (10)	20.0	7.8	158.6	-146.3	5.0
Test (10)	21.2	7.5	158.6	-175.9	0.0
<b>Perch</b>					
Control (10)	20.2	7.9	128.1	-227.5	0.0
Test (10)	20.2	7.7	128.7	-219.7	0.0
<b>Oligochaetes</b>					
Control (14)	21.2	8.0	150.4	-154.5	5.4
Test (14)	21.0	7.9	156.0	-214.3	0.0
<b>Clams</b>					
Control (30)	19.9	8.5	113.8	-272.5	8.3
Test (30)	19.4	8.4	113.3	-271.5	8.3

Table 3. Mean weight, lipid content, and PCB and Zn concentrations in analyzed samples. Standard errors in parentheses. All sample sizes equal 4 except clams (N=8) and oligochaetes (N=2).

Organism and exposure	Weight (g)	Lipid (%)	Contaminants (ug.g dry wt.)	
			PCB	Zn
Fathead minnows				
Preexposure	2.42 (0.16)	8.5 (0.29)	1.0 (0.02)	189.8 (13.36)
Control	2.08 (0.08)	8.1 (0.18)	1.4 (0.04)	227.6* (13.34)
Test	2.06 (0.09)	8.1 (0.08)	45.4* (1.96)	179.2 (3.95)
Yellow perch				
Preexposure	2.10 (0.04)	4.9* (0.27)	1.6 (0.0)	113.5 (6.92)
Control	1.90 (0.03)	3.9 (0.29)	2.0 (0.18)	128.6 (8.15)
Test	1.98 (0.09)	3.6 (0.23)	8.9* (0.73)	118.4 (12.79)
Oligochaetes				
Preexposure	0.94* (0.07)	0.6 (0.07)	0.4 (0.06)	182.9 (24.96)
Control	0.64 (0.04)	0.6 (0.12)	0.5 (0.08)	141.0 (11.47)
Test	0.58 (0.02)	0.5 (0.0)	125.5* (1.88)	171.0 (36.64)
Asiatic clams				
Preexposure	1.05 (0.03)	1.5 (0.23)	0.8 (0.17)	135.1 (9.64)
Control	1.22 (0.04)	1.8 (0.04)	1.1 (0.04)	97.1* (2.55)
Test	1.12 (0.05)	1.9 (0.10)	3.4* (0.14)	117.8 (3.18)

\*Denotes significant difference ( $P < 0.05$ ) from other two treatments based on analysis of variance and Duncan's multiple range test.

efficient accumulators (BF, 4) and clams were the least efficient (BF, of 0.2). Both species of fish accumulated PCB--the BF was 1.4 in fathead minnows and 0.7 in yellow perch.

None of the organisms exposed to test sediments accumulated Zn in any of the bioassays, although we did observe several statistically significant changes in the Zn concentration in organisms from other treatments. Clams exposed to control sediment were significantly lower ( $P < 0.001$ ) in Zn concentration than clams before exposure or those held in test sediments (Table 3). Apparently clams had high levels of Zn in tissues when we received them and some depuration occurred in clean sediments. Fatheads exposed to control sediments had significantly higher ( $P = 0.03$ ) concentrations of Zn than fatheads before exposure or those exposed to test sediments (Table 3). This observation is unexplained.

The weight of oligochaetes and the lipid content of yellow perch both decreased significantly during the bioassay (Table 3). The general trend of decreasing weight and lipid content in both species of fish and in the oligochaetes was expected because the organisms were not fed during the 12-day test.

## Discussion

### System performance

The flow-through bioassay that we evaluated for use in assessing the potential of bioaccumulation from sediments provided conditions allowing for high survival of test organisms. Mortality did not exceed 8.3% in species and half the tests resulted in no mortality. The water flow of 100 mL/min was

sufficient for maintenance of good temperature control and a high oxygen saturation (95%).

The 10-day exposure period, as suggested for evaluating dredged material for ocean discharge (USEPA/CE 1977), was seemingly adequate to assess bioaccumulation potential for organic contaminants with similar chemical characteristics as PCBs. However, it is unlikely that test organisms had reached a steady-state concentration, thus the maximum BFs had probably not been attained (Rubinstein et al. 1983). We did not observe the accumulation of Zn from test sediments in this study--perhaps because the Zn in our test sediments was in a form that is biologically unavailable (Engel et al. 1981); increasing the exposure time would not likely have affected the bioaccumulation of Zn. Rubinstein et al. (1983) showed that increasing the time that marine invertebrates were exposed to contaminated sediments from 10 to 100 days did not result in accumulation of Hg or Cd. Seelye et al. (1982) provided evidence that 10 days is sufficient for measuring bioaccumulation potential of biologically available metals from sediments; they reported accumulation of Zn and several other metals by yellow perch in a 10-day exposure. It thus appears that 10 days is sufficient to measure bioaccumulation potential of metals that are in available form; however not all factors that influence this availability are understood.

The effects of not feeding organisms during the test are not certain. It might be argued that withholding food might result in a loss of PCBs as lipids are mobilized and lipophilic contaminants metabolized. However, if this was occurring to any great extent then organisms exposed to control sediments would have lower PCB levels than unexposed organisms. This loss of PCBs was not

observed. Nevertheless, it is possible that feeding would have enhanced accumulation and perhaps feeding of organisms during the test should be attempted. It may not be successful in tests with fish however due to the high turbidity in aquaria caused by resuspension of fine sediments.

#### Species evaluation

We compared accumulation in two species of fish that have different advantages for use as bioassay test organisms. The yellow perch is widely distributed in the Great Lakes and the young are often found in areas of dredging activity (Barnes 1979). Its commercial and sport fishing value make it an economically important species. On the other hand, the fathead minnow, although also widely distributed in the Great Lakes watershed, is neither as abundant nor as economically important as the perch. It does offer certain other advantages (a) it is routinely available because it is easy to culture, (b) it is tolerant of a wider range of water temperature and dissolved oxygen (Eddy and Underhill 1974), and c) it is widely used as a bioassay organism (Committee on Methods for Toxicity Tests with Aquatic Organism 1975).

Our results show that fathead minnows accumulated higher concentrations of PCBs than did perch. Although this difference was not critical in testing sediments containing high levels of PCBs, the fathead minnow would be the superior test organism for testing sediments with low concentrations of contaminants. Higher lipid levels in fathead minnows (Table 3), as well as behavioral differences between the two species, could account for their greater uptake of PCBs. Fathead minnows were more active at the water sediment interface than were yellow perch, resulting in a greater resuspension of the sediment. This observation is supported by measurements of suspended solids in

tanks of the two species during the bioassay. In control and test sediments, used with fathead minnows suspended solids in control and test sediments were 125.8 and 200.8 mg/L, respectively). In the yellow perch exposure these values were only 23.5 and 27 mg/L.

O. tyrtaeum was shown to be the preferred invertebrate test species because it accumulated PCBs to a greater extent than did the clams. Although this species is not readily available and not a common inhabitant of Great Lakes nearshore areas we feel an oligochaete worm would provide a benthic invertebrate for freshwater testing similar to the polychaete Nereis used in marine sediment evaluation (Rubinstein et al. 1983, Elder et al. 1979). Both Nereis and O. tyrtaeum appear to accumulate organic contaminants readily and are of adequate size to provide ample tissue for analysis.

The Asiatic clam is common in several areas of the country and has been found in western Lake Erie (Scott-Wasilk et al. 1983). However, our results suggest that its suitability as a test species for measuring bioaccumulation is questionable due to several factors: (a) it may cease feeding in certain sediments, (b) it had the lowest BCF of all the species we tested, and (c) the presence of the shell causes confusion as to what to use as an analytical sample. Although Asiatic clams accumulate metals in their shells (Clarke et al. 1979), the ecological and toxicological significance of this metal accumulation is unknown. This leads to uncertainty as to whether shells should be included in the contaminant analysis. Both organic and inorganic analyses are often conducted on samples taken from the same preparation, but the inclusion of shells in the organic analysis could result in analytical problems.

Elder, D. L., S. W. Fowler, and G. G. Polikarpov. Remobilization of sediment associated PCBs by the worm Nereis diversicolor. Bull. Environ. Contam. Toxicol. 21:448-452, 1979.

Engel, D. W., W. G. Sunda, and B. A. Fowler. Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters. In Biological monitoring of marine pollutants, F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg, eds. Academic Press, New York, 1981. pp. 127-144.

May, T. W., and W. G. Brumbaugh. Matrix modifier and L'vov platform for elimination of matrix interferences in the analysis of fish tissues for lead by graphite furnace atomic absorption spectrometry. Anal. Chem. 54:1032-1037, 1982.

Peddicord, R., H. Tatem, A. Gibson, and S. Pedron. Biological assessment of upper mississippi river sediments. Misc. Pap. EL-80-5, U.S. Army Engineers Waterways Experiment Station, Vicksburg, Mississippi, 1980. 82 pp.

Prater, B. L., R. L. Bennett, P. J. Crerar, and R. A. Laskowski-Hoke. An evaluation and refinement of a 96-hour sediment bioassay procedure. (Draft report) EPA 68-01-6471. U.S. Environmental Protection Agency, Chicago, Illinois, 1983. 90 pp.

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- Rubinstein, N. I., C. N. Asaro, and C. Sommers. The effects of contaminated sediments on representative estuarine species and developing benthic communities. In: Contaminants and Sediments, Vol. 1, R.A. Baker, ed. Ann Arbor Science, Ann Arbor, Michigan, 1980. pp. 445-461.
- Rubinstein, N. I., E. Lores, and N. R. Gregory. Accumulation of PCBs, mercury and cadmium by Nereis virens, Mercenaria mercenaria and Palaemonetes pugio from contaminated harbor sediments. *Aquatic Toxicology* 3:249-260, 1983.
- Scott-Wasilk, J., G. G. Downing, and J. S. Lietzow. Occurrence of the Asiatic clam Corbicula fluminea in the Maumee River and western Lake Erie. *J. Great Lakes Res.* 9:9-13, 1983.
- Seelye, J. G., R. J. Hesselberg, and M. J. Mac. Accumulation by fish of contaminants released from dredged sediments. *Environ. Sci. Technol.* 16(8):459-464, 1982.
- Seelye, J. G., and M. J. Mac. Bioaccumulation of toxic substances associated with dredging and dredged material disposal: A literature review. EPA AD-14-F-1-529-0. U. S. Environmental Protection Agency, Chicago, Illinois, 1983. 45 pp.
- USEPA/CE. Ecological evaluation of proposed discharge of dredged material into ocean waters; implementation manual for Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1972). Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1977 (Second Printing 1978). 19 pp. and 8 appendices.

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18. ABSTRACT A bioassay was developed for testing the solid phase of dredged material that measures the survival of organisms, and the bioaccumulation of toxic substances. This bioassay is intended to estimate the bioaccumulation potential from sediments that are not acutely toxic to test organisms, but are suspected of containing persistent contaminants. Two species of fish, <u>Pimephales promelas</u> and <u>Perca flavescens</u> , and two invertebrate species, <u>Octalasion tyrtaeum</u> and <u>Corbicula fluminea</u> were used in the evaluation of this flow-through bioassay. Although not intended as a toxicity test per se, the test enables interpretation of any mortality that may occur during bioassays.		
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20 February, 1985

Dr. Thomas Dillon  
Environmental Laboratory  
U.S. Army Engineers Waterways Experiment Station  
P.O. Box 631  
Vicksburg, Mississippi 39180

Dear Tom,

This is just to inform you officially that I shall be happy to accept your invitation to attend the April Workshop on bioassessment methodologies for the St. Paul District, COE. As you noted in our telephone conversation, I shall submit my pre-meeting information regarding suggested methodologies by 5 March; I shall arrive at the meeting with a full description of the methodology as well as a bibliography of appropriate published information.

I assume you'll send complete information on transportation and hotel arrangements. I look forward to the Workshop, and the catfish.

Sincerely,



Joseph M. O'Connor  
Associate Professor



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4 March, 1985

Dr. Tom Dillon  
Environmental Laboratory  
U.S. Army Engineers Waterways Experiment Station  
P.O. Box 631  
Vicksburg, Mississippi 39180

Dear Tom,

I am submitting, as part of this letter, a list of bioassessment techniques that I consider to be appropriate to the regulatory testing of dredged material for open water disposal.

Prefatory to listing techniques, I must make it clear that, despite opinions to the contrary, I consider bulk chemical analysis of sediment designated for open-water disposal to be a worthwhile exercise, if only to get a handle on the general nature of the toxicants and contaminants present. Given an adequate list of potentially toxic materials in the subject sediment, the regulator has the advantage of knowing, beforehand, the kind of transport and fate processes likely to affect the contaminants in his (her) sediment, and therefore may be able to deal with the whole question of disposal from a position of greater preparedness and awareness. Exhaustive chemical analysis for a variety of classes of potentially toxic contaminants is almost prohibitively expensive if done for all samples; however, I think I can offer somewhat of an alternative (this is to be found as part of the suggested methodology).

Overall, I find it most scientifically pleasing to suggest not individual methodologies, but a protocol for the whole bioassessment process. There are, of course, several different methodologies in the protocol, but by and large, I should think that the people responsible for making the decisions about disposal options will be more comfortable having gone through a series of graded steps, in which the suitability of material for certain kinds of disposal is developed from a "flow chart", if you will.

1. **BULK CHEMICAL ANALYSIS** The first step in the protocol should be bulk chemical analysis, consisting of complete chemical and geochemical analysis of the material slated for disposal. From these analyses one will obtain sufficient data to apply in subsequent evaluations of suitable disposal techniques. Critical data from the chemical analysis, of course, will be the concentrations of key contaminants in the bulk sediment and in sediment elutriate, as well as a complete breakdown on particle size composition and organic content of the waste dredged material.

2. **GENERAL TOXICITY SCREENING** Needless to say, bulk chemical analysis cannot be expected to detect the presence in waste dredged material of toxicants and contaminants that are not detectable by "routine" chemical analysis ("routine"

chemical analyses in this case consisting of metals, chlorinated hydrocarbons, petroleum hydrocarbons, PAHs, phthalates and the like). Therefore, I should like to suggest a second step in the assessment protocol, that being a screening process, in which sequential extracts of the waste material are examined to determine the classes of contaminant present and their potential biological activity (especially such hazardous classes as N- and S- substituted heterocycles, carbonyls, nitrosamines and the like). Using the developing technology of high-precision thin-layer chromatography (HPTLC) it is now possible to screen for a wide variety of chemical classes, their toxicity and their biological activity (as potential mutagens or teratogens) simultaneously, by applying microbial bioassay techniques (Ames assay) directly to the HPTLC plates. (Details are provided below)

3. ESTIMATE OF BIOACCUMULATION FROM MODELS Once chemical classes have been detected for the subject sediment, it is appropriate to derive estimates of maximum possible bioaccumulation for the more routine classes of contaminant (e.g. PCBs, PAHs, dioxins, pesticides, metals, organometallics). Based upon our knowledge of bioaccumulation processes and bioaccumulation modelling techniques, it is recommended that model-derived estimates of potential maximum bioaccumulation be made for those toxicants and contaminants posing the greatest perceived threat to the ecosystem and to human health. Of course, data accumulated in the screening process may have a lot to do with changing our perception of threat; nonetheless, some decision must be made at this stage as to which contaminants pose the greatest potential problem.

The models that may be utilized in this exercise derive from the work of MacFarland, Connolly, Spacie and Hamelink, Mackay, Karickhoff and others. The basic theme behind most of these modelling approaches is to derive an estimate of the concentration of contaminants likely to be present in the sediments, apply a factor for bioavailability (e.g. estimates of organic carbon content and/or particle size distribution) and proceed to estimate equilibrium contaminant body burdens likely to occur in key species based upon octanol-water partition coefficients, or some measure of fugacity.

4. TOXICITY AND BIOACCUMULATION TESTING Following estimates of maximum bioaccumulation for contaminants of concern, the regulator must make a judgement call as to which levels of bioaccumulation and potential toxicity might be "unsuitable". In this regard, it is reasonable to conclude that any contaminant with the potential to accumulate to levels within an order of magnitude of EPA, FDA or WHO standards in aquatic organisms should be examined in greater detail. In this protocol, one would proceed to perform a series of toxicity/bioaccumulation assays in order to determine whether actual toxicity or bioaccumulation approximates that predicted from models. If levels are within a factor of 2-5 of the modelling prediction, then a serious contaminant problem may be concluded to exist.

Toxicity and bioaccumulation testing should include exposure of selected, sensitive benthic or demersal species... including fishes... to representative samples of the sediment, sediment elutriate and water overlying sediment in order to determine both toxicity (lethality) and bioaccumulation of toxic and potentially toxic contaminants.

5. FOOD CHAIN BIOACCUMULATION STUDIES Any evidence of bioaccumulation for subject contaminants may be seen as an indication that food-chain transport is a possibility in the natural ecosystem. Thus, I suggest that studies of

bioaccumulation not be limited to "bioassay" exposure, but also include studies of the type being conducted by Norm Rubinstein, wherein key fishes and their prey are exposed, separately, to contaminants of concern, followed by exposure of the fish to the contaminated food. Such testing enables one to place a reasonable value on the magnitude of food-chain transport, and to determine whether small quantities of the subject contaminant, if placed in the environment, will have the potential to build into large contamination problems in fish populations.

#### DETAILS ON THE HPTLC SCREENING PROCESS

The HPTLC contaminant screening process has been developed in our laboratory as a means for determining rapidly, and inexpensively, whether environmental samples contain toxic or biologically active compounds requiring further study. The procedure is, at this time, specific to organic compounds. Basically, one subjects an environmental sample of sediment, water or aerosol to sequential extraction to methylene chloride, acetone and cyclohexane. The extracts are kept separate, and are run in duplicate on Thin Layer Chromatography plates specifically designed for the separation of non-polar, moderately polar and polar constituents, respectively.

After each type of extract is run on TLC plates (against known standard marker material), one set of plates is covered with a thin layer of nutrient agar, and an "Ames" test is performed directly on the plates. This test consists of plating special mutant cultures of Salmonella typhimurium on the agar. While the bacterial colonies are developing, toxicants and contaminants are diffusing from the TLC plate into the agar, and exerting their activity on the bacteria. Thus, in those portion of the plate where highly toxic compounds are present, the bacteria are killed. In those areas where the toxicant has significant biological activity, mutation rates in the Ames assay are measureable. In those regions where toxicants either do not exist, or are not biologically active, the bacterial colonies grow as expected (i.e., in comparison to control plates).

The advantage of the screening test is that it provides the investigator with a rapid and inexpensive assay of what kinds of toxic or biologically active contaminants may be present in an environmental sample, without the expense of detailed chemical analysis. Once the investigator has evidence that a particular class (known or unknown) of contaminants are present in his or her sample, the spot on the plates may be scraped off and subjected to detailed analysis for determination of compound identification, active components and concentration.

I should stop here, having probably said much more than you wanted to hear. I certainly look forward to the Workshop in April, assuming that it will be as stimulating as the others that I have had the privilege to attend.

Sincerely,

Joseph M. O'Connor



Mr. Norman I. Rubinstein  
U.S. Environmental Protection Agency  
Environmental Research Laboratory  
Narragansett, RI

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CONTAMINANT BIOAVAILABILITY FROM SEDIMENTS

A research proposal to the Corps of Engineers, New York District,  
to develop a screening method for determining  
contaminant bioavailability from sediments

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Research Proposed for EPA/COE Interagency Agreement FY 84  
(Tier I Evaluation of Contaminant Bioavailability)

**BACKGROUND:** At the present time, evaluation of dredged material destined for ocean disposal relies heavily on laboratory bioaccumulation testing. Previous studies have supported this approach by demonstrating that bulk sediment analysis (contaminant concentration in sediment on a dry weight basis) does not accurately reflect bioaccumulation potential of contaminants by representative marine species. The lack of direct correlation between sediment concentration and tissue residues in biota is believed to be related to physico-chemical factors which affect the biological availability of sediment-associated contaminants. However, laboratory testing is an expensive and time-consuming process; consequently, a more cost-effective method is being explored to augment existing procedures for predicting potential bioaccumulation of contaminants associated with material destined for ocean disposal. The approach being considered utilizes basic thermodynamic principles to describe the partitioning of organic contaminants between sediments and biota.

**OBJECTIVE:** To develop a Tier I screening method for predicting the bio-availability and potential bioaccumulation of organic contaminants from sediments. This method will be used by the regulator to identify those sediments which will potentially produce unacceptable concentrations of organic contaminants in tissues of ecologically and commercially important aquatic organisms.

**APPROACH:** A thermodynamic approach for predicting maximum bioaccumulation potential from sediments will be investigated. The approach assumes that for individual organic compounds sediment concentrations based on normalizing factors can be used to predict the thermodynamic maximum concentration accumulated by aquatic organisms. In its simplest form, this relationship can be expressed as follows:

$$[C_s/TOC]=[C_t/L]K$$

Where  $C_s$  = sediment concentration (ug/g dry wt)  
TOC = total organic carbon in sediment  
 $C_t$  = tissue concentration (ug/g dry wt) at steady state  
L = percentage lipid in organism  
K = a constant

This approach views bioaccumulation as a redistribution of contaminants between source materials (i.e., contaminated sediments) and sinks (i.e., organisms) and requires the following assumptions: 1) No kinetic or steric hinderances to the establishment of steady state within the exposure period are present; 2) the concentration accumulated by organisms is lipid dependent; and 3) all of the contaminant associated with sediment is biologically available when normalized for organic carbon.

In order for this approach to have predictive value, K must remain constant over a range of sediment types (varying TOC), species (varying lipid content) and contaminants which reflect a range of partition coefficients. To determine the variability of K over a range of sediments,

species, and compounds, we will examine the available data base and also conduct laboratory bioaccumulation studies to generate the required quality assured information for input into the proposed model.

#### EXPERIMENTAL DESIGN:

Test species, sediments and exposure design will be selected to allow for maximum exchange of organic contaminants between sediments and biota while still considering the biological requirements of the organisms. Because of the need to test a range of contaminated sediments with a variety of organisms, we must first conduct preliminary studies to identify the appropriate sediments, species and exposure design.

Sediment selection will be by mutual consent of ERLN principal investigators and the Corps of Engineers. Sediments selected will contain organic compounds with a range of Log n-octanol/water partition coefficients of at least 3.0 and 6.5, and with low (<2%), medium (~8%), and high (>20%) total organic carbon content. Sediments will be collected from the field by grab sampler, sieved through a 2mm mesh screen to remove large debris, thoroughly homogenized to ensure uniformity, and stored at 4°C prior to experimentation. Sediments will be analyzed for particle size, organic content (TOC), total petroleum hydrocarbons, total oil and grease, moisture content and organic contaminants. The organic compounds analyzed in the sediment samples will include some individual structural isomers of PCB's, nonalkylated PAH's, chlorinated pesticides (e.g., chlordane DDT series) and other compounds as determined by the analyses. These analyses will be conducted using electron capture and flame ionization capillary column gas chromatography and gas chromatography/mass spectrometry. Examples of these analyses are shown (Appendix 1). A complete description of the procedures can be found in Lake et al. (1983). Similar analyses will be conducted on organisms selected for study to allow comparisons of bioaccumulation data on several different compound classes which span a range of Log P. This level of analytical detail is necessary to determine the range of variability of the bioaccumulation model in terms of compound class and Log P. A number of normalizing factors such as TOC, lipid, total PAH's, total oil and grease, and organism wet and dry weight will be examined to determine the utility of these parameters in estimating the bioaccumulation potential of organic compounds by aquatic organisms.

Organism selection will be based on the following: 1) feeding mode (with preference given to organisms which maximize exposure to contaminants); 2) varying lipid content, and 3) compatibility to selected sediment types. Based on species selection, the optimum exposure system will be developed. Following the aforementioned preliminary exercises, a final experimental design will be generated. All testing, holding and acclimation conditions will be in accordance with procedures prescribed by ASTM (1975).

The following example demonstrates a potential experimental approach for infaunal deposit feeding organisms.

Three infaunal organisms will be exposed to bedded sediments in glass aquaria receiving flowing filtered seawater. Organisms will be simultaneously exposed to the three selected sediments in 100 l glass aquaria containing a 5cm layer of homogenized test material. Three aquaria will be set up for each test sediment; a control aquarium containing the three selected species and an uncontaminated substrate will also be monitored. An individual aquarium of each sediment type will be harvested following 10, 28 and 42 days of exposure. Organisms will be placed in uncontaminated (control) sediments for an appropriate time period (to be determined) to purge their intestinal tract of residual test material. Species will then be divided into three replicate groups which will be analyzed for lipids and contaminants of concern. A single control group for each species will be analyzed at this time. Sediment analyses will also be conducted in triplicate at each sampling interval. The range and variability of K will be calculated from the resulting data.

#### PARAMETERS TO BE MEASURED AND CORRESPONDING METHODS

<u>Parameter</u>	<u>Method</u>
<b>SEDIMENT</b>	
particle size	Holme and McIntyre, 1959
TOC	"
moisture	"
total petroleum	Lake et al., 1983
total oil/grease	EPA/CE-81-1, 1981 (Plumb, 1981)
organic contaminants	Lake et al., 1983
<b>ORGANISM</b>	
organic contaminant residue	"
PAH's	"
lipid	Bligh and Dyer, 1959

#### SUMMARY:

This research proposal and analysis of available and unpublished information will provide the data necessary to evaluate the factors that contribute to contaminant bioavailability and affect the variability associated with the K-value. Theoretically, if all of these factors could be accounted for, "K" would be a constant. The degree of confidence with which we can make predictions from the use of this model will be a function of our success in identifying and quantifying the sources of variability. Using statistical procedures (e.g., sensitivity analyses) we can determine and quantify the degree to which each factor contributes to the variability of "K". This is extremely useful since it will allow the prioritization of the factors that must be quantified in order to use the model for decisionmaking. In addition, we will be able to examine the predictive ability of the model as a function of the class of compound (e.g., PAH's, PCB's, etc.) and chemical properties (e.g., log-P, solubility, etc.). Confirmation of this model will provide a level one screening method for predicting maximum contaminant

bioavailability and resultant bioaccumulation from sediments. This will permit regulatory decisions to be made in a more rapid, cost-effective manner than the present sediment-specific biological testing. In particular, this approach will identify both environmentally acceptable sediments and those that contain contaminant levels that require additional testing or regulatory constraints such as post-disposal monitoring.

## REFERENCES

- Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* Vol. 37. pp. 911-917.
- Holme, N.A. and A.D. McIntyre, Eds. 1971. *Methods for the study of marine benthos.* IBP Handbook No. 16. Blackwell Scientific Publications Oxford. 344 pp.
- Lake, J., G. Hoffman and S. Schimmel. 1983. Bioaccumulation of Contaminants from Black Rock Harbor Dredged Material by Mussels and Polychaetes. Technical Report D-83-xx, prepared by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R.I., for the U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.
- Plumb, R.H. Jr. 1981. Procedures for handling and chemical analysis. EPA/COE Technical Committee on Criteria for Dredged and Fill Material. Technical Report No. EPA/CE-81-1.

AQT 00136

**DIETARY ACCUMULATION OF PCBs FROM A CONTAMINATED SEDIMENT SOURCE BY A DEMERSAL FISH (*LEIOSTOMUS XANTHURUS*)\***

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Accumulation and dietary transfer of PCBs from contaminated harbor sediments were studied in a laboratory food chain consisting of sediments, polychaetes (*Nereis virens*) and a predatory fish (*Leiostomus xanthurus*). The study was conducted in two phases to distinguish dietary uptake from PCB accumulation resulting from sediment exposure alone. In phase I fish and polychaetes were separately exposed to field-collected PCB contaminated sediments (5.2 µg/g dry weight as Aroclor 1242 and 1254) in flow-through sea-water systems for 40 days to allow organisms to attain steady state concentrations. In Phase II the dietary fraction of PCB accumulation was determined by selectively feeding exposed and control groups of fish polychaetes having a known PCB body burden. In addition the effect of direct sediment contact on PCB accumulation by *L. xanthurus* was investigated. Results demonstrate that contaminated sediments can serve as a source of PCBs for uptake and trophic transfer in marine systems. Fish exposed to PCB-contaminated sediments and fed a daily diet of polychaetes from the same sediment accumulated more than twice the PCB whole-body residues than fish exposed to similar conditions but fed uncontaminated polychaetes. The dietary contribution of PCBs accounted for 53% of the total body burden measured in fish fed for 20 days, and this percentage appeared to be increasing. Results also indicate that fish isolated from direct contact with PCB-contaminated sediment do not significantly ( $P \leq 0.05$ ) accumulate PCB residues when compared with fish allowed contact with sediment.

**Key words:** PCBs; bioaccumulation; dietary transfer; contaminated sediments

**INTRODUCTION**

Bioaccumulation of organic contaminants by marine organisms occurs through at least three pathways: direct partitioning from the aqueous phase via the gills, integumental sorption, and diet (Swartz and Lee, 1980). Water is the probable medium of exchange for all pathways, and it appears that equilibrium partitioning determines the distribution of organic contaminants between the organism and the

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environment. Of these three routes of uptake, direct partitioning from water across the gills is generally considered to be dominant (Hamelink et al., 1971; Scura and Theilacker, 1977; Macek et al., 1979; Ellgehausen et al., 1980). However, for extremely hydrophobic compounds, such as polychlorinated biphenyls (PCBs), a number of recent studies indicate that diet is a major source of body residues at least for a number of fish species (Bruggemann et al., 1981; Jensen et al., 1982; Pizza and O'Connor, 1983; Thomann and Connolly, 1984).

Because of their hydrophobic nature, PCBs have a strong affinity for particulate material. Consequently, in aquatic systems they are commonly associated with bottom sediments, particularly in urbanized and industrialized areas. Previous studies have demonstrated that a variety of marine organisms including infaunal species can accumulate PCBs from contaminated sediments (e.g., Courtney and Langston, 1978; Fowler et al., 1978; McLeese et al., 1980; Rubinstein et al., 1983). Benthic infauna and epifauna are important food sources for higher trophic organisms. The relative importance of sediments as a contaminant source for the accumulation and transfer of PCBs within marine food webs remains unclear at this time. However, this question becomes pertinent when evaluating the potential impact of dredged material disposal in aquatic systems.

The objective of this study was to determine the extent to which a contaminated sediment (collected from the field) could serve as a source of PCBs for uptake and dietary transfer in a simplified laboratory food chain consisting of sediments, polychaetes and a predatory fish. The predator species selected for study was the spot, *Leiostomus xanthurus*, a commercially important demersal fish which feeds predominantly on polychaetes during its early years (Sheridan, 1979). An infaunal polychaete, the sandworm, *Nereis virens*, was chosen as the prey species. Both of these organisms have been shown to accumulate PCBs from water and sediments (Hansen et al., 1971; McLeese et al., 1980; Rubinstein et al., 1983).

We conducted this study in two phases to determine the relative proportion of PCB residues originating from the spot's diet from residues resulting from environmental exposure alone (direct partitioning via gills and integuments). During Phase I, fish and polychaetes were allowed to establish an 'apparent' steady state concentration. Actual steady state or equilibrium may not be achieved for PCBs within the exposure time-frame (40 days), especially for the more highly chlorinated isomers (Shaw and Connell, 1980). In addition, during Phase I we examined the effect of direct contact with sediments on PCB bioaccumulation potential of spot. In Phase II, we determined the dietary fraction of PCB accumulation by selectively feeding exposed and control groups of fish polychaetes having a known PCB body burden.

Our study was conducted at the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida from November 1982 through January 1983.

## METHODS AND MATERIALS

*Organisms*

Sandworms ( $\bar{x}$  = 6 g) were purchased from the Maine Bait Co., Newcastle, ME, and shipped via air freight to Gulf Breeze. Spot ( $\bar{x}$  = 11 g) were collected (by seine) from Santa Rosa Sound, FL. Both species were acclimated to exposure conditions in the laboratory for at least 2 wk prior to testing.

*Sediments*

Contaminated sediment was collected from Newark Bay, NJ, shipped to Gulf Breeze by refrigerated truck and maintained at 4°C (for about 3 wk) until initiation of the study. Sediment was sieved (2 mm mesh) to remove large debris and macrofauna, thoroughly mixed to insure uniformity, and analyzed for particle size, percentage moisture and percentage organics (EPA/CE, 1981). PCB concentrations (as Aroclor 1242 and 1254  $\mu\text{g/g}$  dry weight) in sediments were measured at the beginning and end of the study. Beach sand (rinsed in sea water) was used as control sediment.

*Phase I. Sediment exposure*

Fish and polychaetes were separately exposed for 40 days to contaminated and control sediments in 100-l glass aquaria (86 × 50 × 25 cm) receiving flowing sea water. Sea water was pumped from Santa Rosa Sound, filtered to 20  $\mu\text{m}$ , and delivered to a headbox in the laboratory; temperature was maintained at  $20 \pm 2^\circ\text{C}$ . Water flowed by gravity from the headbox to a constant head trough where siphons delivered sea water at 30 l/h to aquaria. During the study, salinity ranged from 20 to 30‰ and DO (measured weekly using a YSI Model 57 DO meter) never fell below 5.0 mg/l. Aquaria were set up and designated as follows (Fig. 1):

Tank 1 (exposed worms) - A 4-cm layer (17 l) of contaminated sediment and 200 sandworms.

Tank 2 (exposed fish) - A 4-cm layer of contaminated sediment and 35 spot.

Tank 3 (isolated fish) - A 4-cm layer of contaminated sediment and 20 spot separated from the sediment by a Nitex® screen (1 mm mesh) placed 3 cm above the substrate to isolate fish from direct contact with the sediment.

Tank 4 (control fish) - A 4-cm layer of control sediment and 40 spot.

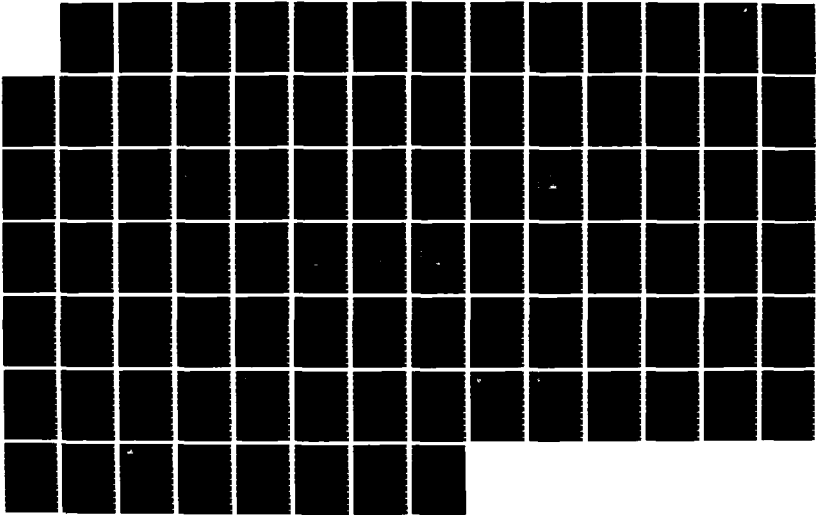
Tank 5 (control worms) - A 4-cm layer of control sediment and 200 sandworms.

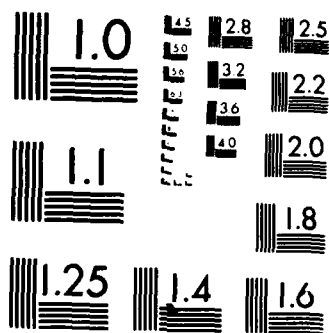
Numbers of fish in aquaria were selected to insure adequate sample size for analysis. Biomass loading in all aquaria did not exceed limits prescribed by ASTM (1983).

A sediment trap was placed in the effluent line of Tank 2 to collect sediment resuspended by the swimming activity of the fish. This material was periodically

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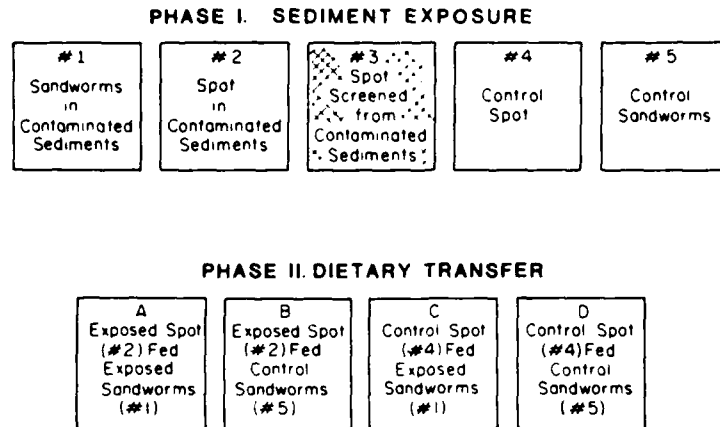


Fig. 1. Exposure design for spot and sandworms in 100-l aquaria during Phase I and Phase II.

returned to Tank 2. During Phase I, fish and polychaetes were fed a maintenance diet of flake food (Tetra SM80, Tetra Werke, F.R.G.) at approximately 2% of body weight per day. The flake food was analyzed and found to be free of PCBs (detection limit  $\geq 0.005 \mu\text{g/g}$ ).

Prior to sediment exposure (Phase I), 3 fish and 3 polychaetes were collected from holding aquaria and analyzed (whole body) for background concentrations of PCBs. Fish collected from Tank 2 (sediment exposed,  $n=3$ ) and Tank 4 (control) were analyzed for PCBs following 10 and 40 days of exposure to test sediments; fish from Tank 3 (sediment isolated) were analyzed on day 10 and 35. Polychaetes ( $n=3$ ) from Tank 1 (sediment exposed) and Tank 5 (control) were analyzed for PCBs on days 10, 20 and 35. Fish and polychaetes were placed in uncontaminated flowing sea water for 24 h prior to tissue analysis to evacuate their intestinal tracts.

#### *Phase II. Sediment and dietary exposure*

Following exposure for 40 days to sediment, fish and sediment from Tank 2 were equally divided into two aquaria so that exposure conditions were maintained identical to Phase I. Control fish (Tank 4) were divided similarly. During the last 2 wk of Phase I, the diet of the spot was gradually adjusted to include increasing portions of uncontaminated (control) sandworms from Tank 5. At the end of this acclimation period, fish were feeding voraciously on polychaetes. For Phase II, aquaria were redesignated as follows (Fig. 1):

Tank A - Contaminated sediment and 13 spot (from Tank 2) fed a daily ration of contaminated sandworms (from Tank 1).

Tank B - Contaminated sediment and 13 spot (from Tank 2) fed a daily ration of uncontaminated sandworms (from Tank 5).

Tank C - Control sediment and 15 spot (from tank 4) fed a daily ration of contaminated sandworms (from Tank 1).

Tank D - Control sediment and 15 spot (from tank 4) fed a daily ration of uncontaminated sandworms (from Tank 5).

Polychaetes in exposed and control aquaria were maintained as in Phase I.

Daily food rations for all fish during Phase II were estimated at 10% of body weight. Sandworms were collected daily from aquaria and cut into pieces small enough for ingestion by spot. Daily samples of contaminated and control sandworms used as food were composited into weekly samples, homogenized and analyzed for PCBs in triplicate. Sandworms used as food were not purged.

Fish in all aquaria consumed their respective food ration quickly. Excess food was not observed in aquaria following feeding. Five fish were sampled for chemical analysis from each aquaria after 10 and 20 days of feeding (day 50 and 60 of sediment exposure). Fish were placed in flowing uncontaminated sea water and not fed for 24 h to evacuate the digestive tract prior to PCB analysis.

Comparisons of PCB body burdens between treatments were statistically determined by analysis of variance using the General Linear Model procedure and Duncan's multiple range test (SAS, 1982). Differences were considered significant at  $P \leq 0.05$ .

#### CHEMICAL ANALYSIS

##### *Tissues*

Whole fish and polychaetes were cut into small pieces and then slurried with an equal weight of distilled water using a polytron (Brinkman, Model PCU-2 with a PT-10 generator). Subsamples (maximum slurry weight of 16 g) were homogenized with aliquots of 10.5 and 5 ml of acetonitrile. After each homogenization, the samples were centrifuged and the supernatant was decanted. Acetonitrile extracts (20 ml) were combined with 75 ml of 2% aqueous  $\text{Na}_2\text{SO}_4$  and extracted three times with 10 ml hexane. The hexane extracts were reduced to 1 to 2 ml by gentle warming under a stream of dry nitrogen. The concentrates were then transferred to a Florisil column for cleanup.

##### *Sediments*

Sediments were slowly air dried at room temperature to 3 to 5% moisture content and then ground to a fine powder using a high speed blade mill. Subsamples of up to 4 g were then extracted by the Soxhlet method of Bellar et al. (1980). Extracts were reduced to a volume of 1 to 2 ml for Florisil cleanup.

##### *Cleanup*

A 9-mm (o.d.) column was packed with 4 g of activated Florisil and topped with

25 mm of anhydrous  $\text{Na}_2\text{SO}_4$ . The column was pre-eluted with 10 ml hexane (not collected) and the 1 to 2 ml samples immediately were introduced and eluted with several washes of hexane (total of 10 ml). This was followed by additional elutions with 10 ml hexane and 10 ml 1% diethylether in hexane. Eluates originating from sediment samples were reduced to 5 ml and tumbled with 0.1 to 0.3 ml metallic Hg for 1 h to remove sulfur interferences. All samples were then reduced to a final volume of 1 ml for analysis by gas chromatography.

### *Analysis*

Analysis was performed on a Hewlett-Packard 5710 gas chromatograph with an electron-capture detector operated at  $300^\circ\text{C}$  and a 1.8-m glass column (4mm ID  $\times$  6 mm OD) packed with 3% OV-101 on 80/100 mesh-Supelcoport maintained at  $200^\circ\text{C}$  for Aroclor 1242 and at  $220^\circ\text{C}$  for Aroclor 1254. The carrier gas was 10% methane in argon at a flow-rate of 60 ml/min.

PCB concentrations were measured by the method of Webb and McCall (1973). The reference standard, obtained from U.S. EPA, Analytical Standards Branch, Cincinnati, OH, was described by Sawyer (1978). Only Aroclors 1242 and 1254 were quantified. Recoveries from spiked samples averaged 86% ( $n = 35$ ,  $\text{SD} = 11.2$ ). Concentrations reported were not corrected for percentage recovery. Instrument detection limits for sediments (dry weight) and tissues (wet weight) were 5 ng PCB/g.

### RESULTS AND DISCUSSION

Test sediment contained 21.8% total volatile solids and 70% moisture. Particle size distribution was 0% sand, 88% silt and 12% clay. A net loss of PCBs in sediment was observed during the exposure period. Initial sediment concentrations which averaged  $5.68 \pm 0.51 \mu\text{g PCB/g}$  ( $n = 5$ , dry weight) dropped to  $4.13 \pm 0.51 \mu\text{g PCB/g}$  ( $n = 5$ ) at the termination of the study. Test sediment was not acutely toxic to fish or polychaetes. No mortality was observed in spot and few polychaetes died ( $< 2\%$ ) during the test period.

#### *Phase I*

Phase I was designed to expose fish to environmentally realistic concentrations of PCBs prior to dietary exposure and to provide a PCB contaminated food source. Whole body concentrations of PCBs in spot and sandworms exposed to contaminated sediments reached an apparent equilibrium concentration during Phase I (Table I). In previous studies conducted with these species, steady state concentrations of PCBs were attained within 40 days of exposure (Hansen et al., 1971; McLeese et al., 1980; Rubinstein et al., 1983). Significant differences in PCB residues between control and exposed treatments were detected in Phase I for both

TABLE I

Phase I - PCB whole body residues in sandworms and spot ( $\mu\text{g/g}$  wet wt.)

Day 0 background		Tank no.	Sample interval		
<b>Sandworms</b>					
	0.01	1 (Sediment exposed)	Day 10	Day 20	Day 35
	0.01		0.17	0.28	0.21
	0.01		0.23	0.21	0.22
	0.01		*	0.13	0.20
$\bar{x}$	0.01	$\bar{x}$	0.20	0.20	0.21
SD	0.00	SD	-	0.08	0.01
		5 (Control)	0.04	0.01	0.02
<b>Spot</b>					
		2 (Sediment exposed)	Day 14	Day 40	
	0.07		0.38	0.29	
	0.10		0.38	0.29	
	0.07		0.31	0.35	
$\bar{x}$	0.08	$\bar{x}$	0.36	0.31	
SD	0.02	SD	0.04	0.04	
		3 (Sediment isolated)	Day 10	Day 35	
			0.19	0.06	
			0.15	0.11	
			0.09	0.08	
		$\bar{x}$	0.14	0.08	
		SD	0.05	0.02	
		4 (Control)	Day 10	Day 40	
			0.12	0.07	
			0.10	0.06	
			*	0.07	
		$\bar{x}$	0.11	0.07	
		SD	-	0.01	

\*Sample lost.

fish and polychaetes. At the end of Phase I PCB body burdens averaged  $0.31 \mu\text{g/g}$  (wet weight) for fish and  $0.21 \mu\text{g/g}$  (wet weight) for polychaetes exposed to contaminated sediment (Table I).

Fish isolated from direct contact with sediment (Tank 3) contained significantly less PCB than fish in contact with sediment (Tank 2). PCB concentrations in fish isolated from test sediment for 35 days were indistinguishable from control fish (Table I, Fig. 2).



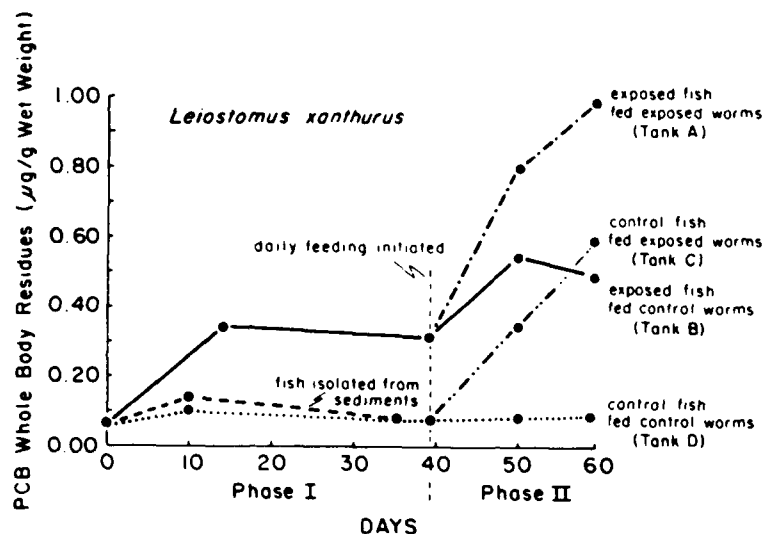


Fig. 2. Average PCB whole body residues ( $\mu\text{g/g}$  wet weight) in spot during Phase I ( $n = 3$ ) and Phase II ( $n = 5$ ).

Halter and Johnson (1977) showed that a freshwater fish (fathead minnow, *Pimephales promelas*) in direct contact with contaminated sediment accumulated PCB residues at six times the rate of fish screened from direct contact with sediments. Our data support the contention that physical isolation of contaminated sediment can effectively reduce the availability of PCBs for bioaccumulation by water column organisms (O'Connor and O'Connor, 1983; Brannon et al., 1984). However, it is important to note that due to our use of a flow-through sea-water design the PCB distribution in our exposure system may not reflect PCB partition equilibrium between sediment and overlying water. Although this may obscure the ultimate contribution of water-mediated uptake observed, we feel that: (1) flow-through conditions are more simulative of open ocean disposal sites where mixing and water movement over the bottom are substantial; (2) static conditions are unacceptable for bioaccumulation studies in that secondary uptake (resulting from depuration) cannot be readily quantified, and (3) flow through conditions are preferable to meet the life support requirements of test organisms in contact with anaerobic sediments for extended periods of time.

#### Phase II

Significant differences in PCB whole body residues in spot were detected between contaminated and control feeding regimes during Phase II (Table II). Fish exposed to contaminated sediments and fed a daily diet of polychaetes from the same

TABLE II

Phase II - PCB whole body residues ( $\mu\text{g/g}$  wet wt.) in spot

Days of exposure	Days of feeding		Tank A	Tank B	Tank C	Tank D
50	10		0.57	0.62	0.33	0.08
			0.60	0.37	0.34	0.11
			1.04	0.60	0.33	0.02
			0.88	0.58	0.29	0.10
			0.89	0.62	0.35	*
		<i>x</i>	0.80	0.56	0.33	0.08
		SD	0.20	0.11	0.02	0.04
60	20		0.75	0.56	0.33	0.08
			0.96	0.33	0.57	0.10
			0.91	0.64	0.64	0.11
			1.54	0.56	0.58	0.10
			*	0.52	0.55	0.11
		<i>x</i>	1.04	0.48	0.60	0.10
		SD	0.35	0.13	0.06	0.02

\*Sample lost.

sediments for 20 days accumulated more than twice the PCB residues than sediment exposed fish fed control polychaetes (Fig. 2). Average ( $n = 5$ ) PCB body burdens on day 60 (20 days of feeding) for fish in Tank A was  $1.04 \pm 0.13 \mu\text{g/g}$  while fish in Tank B (sediment exposure only) measured  $0.48 \pm 0.13 \mu\text{g/g}$ . PCB concentrations of fish in Tank B increased during Phase II (Fig. 2). This increase could be attributed to resuspension of sediment (remobilization of PCBs) which took place when we divided sediment and fish into two groups following Phase I.

Sandworms exposed to contaminated sediment provided the only source of PCBs for control fish during Phase II. Average PCB whole body residues measured in unpurged sandworms used as food during Phase II was  $0.49 \pm 0.09 \mu\text{g/g}$  wet weight ( $n = 8$ ) for sediment exposed, and  $0.01 \pm 0.004 \mu\text{g/g}$  ( $n = 8$ ) for control treatments (Table III). Average ( $n = 5$ ) PCB whole body residues in control fish maintained on a diet of contaminated polychaetes for 20 days measured  $0.60 \pm 0.06 \mu\text{g/g}$  wet weight, while control fish fed control polychaetes during the same period contained  $0.10 \pm 0.02 \mu\text{g/g}$  wet weight (Table III).

On day 60 the PCB dietary contribution to whole body residues in fish was still increasing (Fig. 2) and rates of uptake were similar between exposed fish ( $0.030 \mu\text{g PCB/g}$  per day) and control fish ( $0.025 \mu\text{g PCB/g}$  per day) fed contaminated polychaetes. A comparison of the regressions for PCB whole body residue vs. time for these two treatments (Tank A and Tank C) showed no significant difference

TABLE III

PCB concentrations ( $\mu\text{g/g}$  wet wt.) in weekly food composites (sandworms, gut unpurged)

Treatment	Week 1	Week 2	Week 3	
Exposed	0.45	0.58	0.50	
	0.46	0.59	0.34	
	0.47	*	0.43	
	-----			
	$\bar{x}$	0.46	0.59	0.43
	SD	0.01	-	0.08
Control	0.02	0.02	0.01	
	0.01	0.02	0.01	
	0.01	0.02	*	
	-----			
	$\bar{x}$	0.01	0.02	0.01
	SD	0.00	0.00	-

\*Sample lost.

( $P \leq 0.05$ ) in the slopes of the lines (Fig. 2). Fish in all treatments grew during this study period. Increases in wet weight of individual fish during phase II averaged  $2.83 \pm 1.28$  g ( $n = 16$ ) for all treatments.

Our results demonstrate that contaminated harbor sediments can serve as a source of PCBs for accumulation and dietary transfer by sandworms and spot. Following 20 days of feeding the dietary contribution of PCBs accounted for 53% of the total body residue measured in spot, and this percentage appeared to be increasing (Fig. 2). This observation is in agreement with previous findings by Thomann (1981), Jensen et al., (1982) and Pizza and O'Connor (1983) who identify diet as the major source of PCBs for a variety of predatory fish species. Although the relative contribution of direct partitioning across the gills is extremely high for organic compounds and produces very large bioconcentration factors, one must consider the ultimate distribution of hydrophobic compounds in the marine environment. These compounds (of which PCBs serve as an excellent model) have very low solubilities and very high partition coefficients. Consequently, little of the compound would seem to be available for aqueous uptake compared to the amount of compound which is associated with particulate organic material that can serve as a potential food source for infaunal and epibenthic food webs.

## ACKNOWLEDGEMENTS

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et al. (1984) a research report prepared for the Environmental Laboratory of the U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, under Interagency Agreement No. A-21-F-2-5770 between the Environmental Research Laboratory, Gulf Breeze, FL, and the Waterways Experiment Station, Vicksburg, MS. The authors wish to thank Mr. J. Mansky for supplying test sediment, Ms. T. Dunn for technical assistance, Ms. B. Jackson for editing, and Ms. J. Seites for word processing.

#### REFERENCES

- ASTM, 1983. Proposed standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. Draft no. 7, J.L. Hamelink and J.G. Eaton, co-chairpersons. E-47.01.
- Bellar, T.A., J.J. Lichtenberg and S.C. Lonneman, 1980. Recovery of organic compounds from environmentally contaminated bottom material. In: Contaminants and sediments, Vol. 2, edited by R.A. Baker, Ann Arbor Science Publishers, Ann Arbor, MI, pp. 57-70.
- Brannon, J.M., R.E. Hoeppe, T.C. Sturgis, I. Smith and D. Gunnison, 1984. Effectiveness of capping in isolating dutch kills sediment from biota and the overlying water. U.S. Army Corps of Engineer Waterways Experiment Station Technical Report, in publication, 48 pp.
- Bruggemann, W.A., L.B.J. Martron, D. Kooiman and O. Hutzinger, 1981. Accumulation and elimination kinetics of di-, tri- and tetra chlorobiphenyls by goldfish after dietary and aqueous exposure. *Chemosphere* 10, 811-832.
- Courtney, W.A. and W.J. Langston, 1978. Uptake of polychlorinated biphenyl (Aroclor 1254) from sediment and from seawater in two intertidal polychaetes. *Environ. Pollut.* 15, 303-309.
- Elder, D.L., S.W. Fowler and G.G. Polikarpov, 1979. Remobilization of sediment-associated PCB's by the worm *Nereis diversicolor*. *Bull. Environ. Contam. Toxicol.*, 21, 448-452.
- Ellgehausen, H., J.A. Guth and H.O. Esser, 1980. Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food chains. *Ecotoxicol. Environ. Safety* 4, 134-150.
- EPA/CE, 1981. Procedures for handling and chemical analysis of sediment and water samples. Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Fowler, W., C.G., Polikarpov, D.L. Elder and J.P. Villeneuve, 1978. Polychlorinated biphenyls: accumulation from contaminated sediments and water by the polychaete *Nereis diversicolor*. *Mar. Biol.* 48, 303-309.
- Halter, M.T. and H.E. Johnson, 1977. A model system to study the desorption and biological availability of PCB in hydrosols. In: *Aquatic Toxicology and Hazard Evaluation*, edited by F.L. Mayer and J.L. Hamelink. American Society for Testing Materials, ASTM STP 634, 178.
- Hamelink, J.L., R.C. Waybrand and R.C. Ball, 1971. A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. *Trans. Am. Fish. Soc.* 100, 207-213.
- Hansen, D.J., P.R. Parrish, J.I. Lowe, A.J. Wilson and P.D. Wilson, 1971. Chronic toxicity, uptake and retention of Aroclor 1254<sup>®</sup> in two estuarine fishes. *Bull. Environ. Contam. Toxicol.* 6, 2, 113-119.
- Jensen, A.L., S.A. Spigarelli and M.M. Thommes, 1982. PCB uptake by five species of fish in Lake Michigan, Green Bay of Lake Michigan, and Cayuga Lake, New York. *Can. J. Fish. Aquat. Sci.* 39, 700-709.
- Macek, K.J., S.R. Petrocelli and B. Sleight, 1979. Considerations in assessing the potential for, and significance of, biomagnification of chemical residues in aquatic food chains. *Aquatic Toxicology* ASTM STP 667, edited by L.L. Marking and R.A. Kimerle, American Society for Testing and Materials, pp. 251-268.

- McLeese, D.W., C.D. Metcalfe and D.S. Pezzack, 1980. Uptake of PCB's from sediments by *Nereis virens* and *Crangon septemspinosa*. Arch. Environ. Contam. Toxicol. 9, 507-518.
- O'Connor, J.M. and S.G. O'Connor, 1983. Evaluation of capping operations at the experimental mud dump site N.Y. bight apex. Technical Report D-83-3, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS, 69 pp.
- Pizza, J.C. and J.M. O'Connor, 1983. PCB dynamics in Hudson River striped bass. II. Accumulation from dietary sources. Aquat. Toxicol. 3, 313-327.
- Rubinstein, N.I., E. Loes and N. Gregory, 1983. Accumulation of PCB's, mercury and cadmium by *Nereis virens*, *Mercenaria mercenaria* and *Palaemonetes pugio* from contaminated harbor sediments. Aquat. Toxicol. 3, 249-260.
- Rubinstein, N.I., W.T. Gilliam and N.R. Gregory, 1984. Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish species. Technical Report in publication, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS, 25 pp.
- SAS, 1982. Users guide: statistics. SAS Institute, Inc., Gary, NC, 582 pp.
- Sawyer, L.D., 1978. Quantitation of polychlorinated biphenyl residues by electron capture gas-liquid chromatography: collaborative study. J. Assoc. Off. Anal. Chem. 6, 282-291.
- Schneider, R., 1982. Polychlorinated biphenyl (PCB's) in cod tissues from the Western Baltic: significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. Meresforsch 29, 69-79.
- Scura, E.D. and G.H. Theilacker, 1977. Transfer of the chlorinated hydrocarbon PCB in a laboratory food chain. Mar. Biol. 40, 317-325.
- Seelye, J.G., R.J. Hesselberg and M.J. Moc, 1982. Accumulation by fish of contaminants released from dredged sediments. Environ. Sci. Technol. 16, 459-464.
- Shaw, G.R. and D.W. Connel, 1980. Relationship between steric factors and bioconcentration of polychlorinated biphenyls (PCB's) by the sea mullet (*Mugil cephalus*). Chemosphere 9, 731-743.
- Sheridan, P.F., 1979. Trophic resource utilization by three species of sciaenid fishes in a northwest Florida estuary. Northwest Gulf Sci. 3, 1-15.
- Swartz, R.C. and H. Lee II, 1980. Biological processes affecting the distribution of pollutants in marine sediments, Part. I: accumulation, trophic transfer, biodegradation and migration. In: Contaminants and sediments, Vol. I, edited by R.A. Baker, Ann Arbor Science Publishers, Ann Arbor, MI, pp. 553-555.
- Thomann, R.V., 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38, 280-296.
- Thomann, R.V. and J.P. Connolly, 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18, 65-71.
- Webb, R. and A. McCall, 1973. Quantitative PCB standards for electron capture gas chromatography. Chromatogr. Sci. 11, 366.

Dr. John Scott  
Science Applications International Corporation  
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March 4, 1985

Dr. Thomas Dillon  
Department of the Army  
Waterways Experiment Station, Corps of Engineers  
PO Box 631  
Vicksburg, MI 39180

Dear Tom,

Enclosed is a short description of the type of testing we have been doing here at ERL-M. As you know, these cover a wide range of tests from the subcellular to the community. I've not discussed residues since I think Norm will cover that. Also note a short blurb on biomonitoring as the last piece.

I'll call you in the next few days to go over details of my discussion.

Best regards,

K. John Scott  
Program Manager

Enclosure

KJS: ek

**Science Applications, Inc.** One Washington Street, P.O. Box 509, Newport, Rhode Island 02840 (401) 847-4210

**Other SAI Offices:** Albuquerque, Ann Arbor, Arlington, Atlanta, Boston, Chicago, Huntsville, La Jolla, Los Angeles, Palo Alto, Santa Barbara, Sunnyvale, and Tucson.

MARINE BIO-ASSESSMENT  
METHODOLOGIES:  
A TIERED APPROACH

SHORT TERM/ACUTE ENDPOINTS:

The purpose of this testing is to determine the general range of toxicity of the dredged material to the organisms of interest. The results apply only to the immediate impact of the disposal operation at the site. Solid phase and suspended phase tests should be conducted and exposures should include a control sediment, mixed with the test sediment if appropriate. Test duration is typically 4 to 10 days depending on the organism. For marine testing the species of choice are crustaceans, either mysids or amphipods, with a preference for at least one infaunal organism. The endpoint is mortality.

LONG TERM/CHRONIC ENDPOINTS:

The long term tests provide information on more sub-lethal effects, and they attempt to predict the consequences of dredged material disposal on the benthos during the period following the immediate disposal activity. These tests make predictions at the individual and the population or community levels of organization.

Testing on individuals have used the marine mussel and infaunal polychaetes. Test duration is from 10 days, for the polychaete, and up to 28 days for the mussel. Again, the exposure may be either solid phase or suspended phase dredged materials; both are used in the polychaete testing while the suspended phase is most appropriate for the filter-feeding mussel. The endpoints that are measured, with a short description, are as follows:

SCOPE FOR GROWTH (SFG) - This index is an instantaneous physiological measure of general health of the organism. It integrates measures of respiration rate, food assimilation efficiency and excretion rate, to predict the energy available for production after accounting for routine metabolic costs.

HISTOPATHOLOGY - This endpoint provides qualitative and quantitative assessment of pathological changes induced by exposure to dredged materials.

ADENYLATE ENERGY CHARGE (AEC) - AEC is an indicator of stress and measures the amount of energy available to an organism from the adenylate pool. Measurements are made of the three adenine nucleotides: ATP, ADP and AMP. The index ranges from 1.0, when all the adenylate is ATP, to 0.0 when all the adenylate is AMP.



SISTER CHROMATID EXCHANGE (SCE) - This technique measures the occurrence of genetic damage through mutagenic activity. It predicts such long term consequences as loss in viability of the individual and a reduction in ecological fitness of the population.

To date, SFG and Histopathology have been the more effective measures of responses to dredged materials, both in the laboratory and in the field. AEC and SCE are more "state of the art" at this time and are presently being evaluated for application on a more routine basis.

Predictions of population level effects have exposed mysids and amphipods to dredged materials for periods of 28 to 60 days. The measured endpoints are population survival, growth and reproduction. These endpoints have been integrated, using standard demographic models, to predict the effects of suspended dredged materials on "r", the biotic potential of the population. Since, from a regulatory perspective, the population is the minimum ecological unit to be protected, these tests provide very realistic predictions of effects.

Finally, community responses to dredged materials can be measured in the laboratory using recolonization studies. These tests attempt to predict the recovery rate of the disposal mound and the surrounding area.

The techniques just described are those that have been used at ERL-Narragansett in the EPA-CDE Field Verification Program. A component of sediment testing that has not been employed in the FVP is a behavioral response, primarily sediment avoidance. These tests have been successfully used with crustaceans, polychaetes and bivalves. The avoidance response, however, is often difficult to interpret because the organisms are not truly exposed to the sediment for any great length of time.

## BIOMONITORING

Laboratory assessment techniques are capable of predicting eventual effects in the field. To determine the accuracy of these predictions, a monitoring program should be implemented. The primary goals of a monitoring program are to determine:

1. The recovery rate of the disposal mound as compared to background.
2. The effects on the benthic environment, off site.
3. The potential for dispersal of the dredged material as a result of aperiodic climatic events.

The dispersion of dredged materials can be assessed by examining tissue residues of contaminants of interest in the resident fauna. In addition, standard quantitative community analysis will provide information on benthic community effects and recovery rates of the disposal mound.

To effectively sample the disposal area, however, mound morphology and location should be accurately described. Also, measures of natural habitat variability will greatly increase the cost effectiveness of the sampling design. Precision bathymetry and positioning systems can accurately describe sample locations, while the REMOTS interface camera can be used to assess habitat variability.

Mr. Tim Ward  
Erco: A Division of Enseco, Inc.  
Cambridge, MA

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February 5, 1985


Dr. Tom Dillon  
Environmental Laboratory  
Department of the Army  
Waterways Experiment Station  
Corps of Engineers  
Vicksburg, Mississippi 39180

Dear Tom:

I have enclosed a summary of bioassessment techniques that I have used during the last seven years. I have spent that time conducting studies in response to methods specified by various COE Districts or private clients whose guidance has come from the districts. As a result, my experience has been pretty "cookbook," and with some modification has only involved methods outlined in the EPA/COE implementation manual. We have performed dredged material studies from more than 50 locations along the east coast of the United States, and I have tried to include all the modifications of the procedure that we have experienced, and to mention all the points of discussion that we have encountered.

I hope this information is helpful, and I look forward to attending the workshop.

Sincerely,

  
Timothy J. Ward  
Director  
Aquatic Toxicology  
Laboratory

TJW: sbm  
Encl.

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## DREDGED MATERIAL BIOASSESSMENT TECHNIQUES

The most significant bioassessment technique used at ERCO to date is the three-phase bioassay/bioaccumulation method defined by the U.S. EPA and COE (1977). This method was, in some cases, vaguely defined and so has been widely modified. The following summary of the technique lists various options that have been employed. The technique sorts itself easily into five categories (sample collection, liquid and suspended particulate phase bioassays, solid phase bioassays, bioaccumulation analyses and interpretation of results).

### Sample Collection

Samples are collected with a noncontaminating sampling device and stored in appropriate containers. There are four major areas open to further definition:

1. Sampling device. Three major types of samples have routinely been collected: 1) grab samples which are collected to a depth of approximately 0.5 meters. This technique is quick, cost-effective, minimizes disturbance of surface sediments and collects the most recently deposited (and potentially contaminated) sediments; 2) core samples to refusal. Refusal is normally defined as the depth to which a free-falling gravity corer will penetrate, and normally occurs at a layer of sand or stones. This technique is moderately complex, causes some disturbance of sediment, and normally results in samples which represent the top 1-3 meters; 3) core samples to project depth, or the depth to which dredging will remove sediment. This technique is very costly and complex, and normally results in extensive disturbance of sediment, but can be used to obtain cores of 10 meters or more.

2. Number of Sample Sites. Major questions arise concerning both the number and location of samples. Enough samples must be collected to adequately characterize the site, but economic constraints must be considered. Should sample sites be arbitrarily located to include important areas or "hot spots," or should standard randomization techniques be employed? A widely accepted technique is to collect a relatively large number of samples and composite them for laboratory analysis.
3. Storage Method. Because relatively small sample volumes are required for chemical analyses of sediment, glass or teflon containers are used to prevent contamination. The large volumes required for laboratory bioassessment make necessary a compromise. At ERCO, samples are stored in acid-soaked linear polyethylene buckets (1-5 gallons in volume).
4. Sample Storage Time. All samples are stored in completely filled, sealed containers at 20°C. Storage times are normally less than 2 weeks, but storage for up to 30 days has been allowed by the New York District, COE.

#### Liquid and Suspended Particulate Phase Bioassays

Samples are combined in a 1:4 ratio by volume with water (from the disposal site or equivalent), mixed for 30 minutes, allowed to settle for 1 hour, and the supernatant decanted. This supernatant is the suspended particulate phase. The liquid phase is obtained by filtering the supernatant through a 0.45-micron filter. Acute toxicity tests (bioassays) are then conducted for 96 hours with three appropriate organisms and four concentrations of each phase (0, 10, 50, and 100 percent). Ninety percent survival of control organisms is required, or a test is repeated.

Methods for bioassays are well defined in the U.S. EPA and COE manual (1977). Several modifications of this technique have been encountered including: 1) the elimination of all liquid and suspended particulate phase tests on the grounds that they are "doomed to succeed," i.e., all historically tested sediments have failed to uncover any serious toxicity; 2) elimination of the liquid phase tests on the grounds that since the suspended particulate phase contains the liquid phase, the testing of both is redundant; 3) elimination of the 50 percent and 10 percent concentrations unless toxicity is noted at the 100 percent concentration level.

Areas that offer a variety of options include:

1. Dilution Water. While water collected at the disposal site has been used, practical considerations for collection and appropriate storage of the volumes of seawater necessary for biological testing usually make it impractical. The use of some standard water is, therefore, necessary (water from an alternative source or laboratory prepared "synthetic" water).
2. Organism Selection. While locally important organisms are desired, a compromise must be struck to allow organisms which can survive and prosper under the laboratory conditions to be used. A more likely approach would be to establish a list of standard organisms for each locality. The number and type of organisms used (usually three dissimilar species) must be chosen carefully to assure adequate protection of the ecosystem.

### Solid Phase Bioassays

Three species of benthic organisms are exposed to sediment from the proposed disposal site, control sediment, and a combination of disposal site sediment and dredged material for a period of 10 days. Thirty-eight liter aquaria which hold 20 liters of media are used to simultaneously expose the three species. A 30-mm layer of disposal site sediment (sieved to remove live organisms) is placed in each noncontrol aquaria and a 30-mm layer of control sediment is placed in each control aquaria, and water is added. Burrowing benthic organisms are allowed 48 hours to establish burrows and then a 15-mm layer of control sediment is added to each of five control aquaria, a 15-mm layer of disposal site sediment is added to each of five disposal site (reference) aquaria, and a 15-mm layer of dredged material is added to each of five test aquaria which already contain a 30-mm layer of disposal site sediment. Epibenthic test organisms are added to each aquaria immediately after final addition of sediment. The test continues for 10 days during which water is replaced and sediment is left undisturbed. Dead organisms are removed when observed and behavior of organisms is noted. After 10 days, sediment from each aquaria is sieved and the number of live organisms is noted. All survivors are placed in sediment-free aquaria for 48 hours and frozen at  $-15^{\circ}\text{C}$  in solvent-rinsed foil or acid-soaked polyethylene. If less than 90 percent survival occurs in the control aquaria, the test is repeated. Areas of variation of technique include:

1. Dilution water - disposal site, alternative site, or laboratory prepared.
2. Replacement of seawater (75 percent of volume every 48 hours), or flow-through (six volume exchanges per 24 hours) methods have both been used successfully.



Flow-through techniques may represent a worse condition; since sediment is not added, the exchange of water actually may serve to dilute contaminants and particulates.

3. Control sediment, which is used solely to determine the quality of test organisms, can be collected from various locations chosen by the laboratory or specified by the regulatory authority.
4. Selection of organisms - locally important or standard.
5. Compatibility of test organisms - all species of animals can be simultaneously exposed in a single aquaria (if they are compatible and biomass considerations can be satisfied), or each exposed in a separate aquaria (less cost effective).
6. Temperature - a single temperature is usually used for each location, but seasonal variation can be considered.
7. Depuration - a 48-hour exposure of test organisms to sediment-free water to empty their guts may allow some loss of important compounds from tissues. One option would be immediate freezing of tissues and dissection of digestive tracts, although this technique is very time consuming and expensive.

#### Bioaccumulation Analyses

Tissues of surviving organisms from the solid phase bioassay have been analyzed for up to six chemical constituents: DDT, PCBs, aliphatic petroleum hydrocarbons, aromatic petroleum hydrocarbons,

mercury and cadmium. Organic constituents are extracted and analyzed by gas chromatography. Inorganic constituents are analyzed by graphite furnace or cold-vapor atomic absorption spectrophotometry following tissue digestion. Variations include:

1. Elimination or alteration of the chemical constituents to be studied. If it is known (from bulk or elutriate analyses, or other historical data) that a particular constituent is not present in the sediment, that analysis can be eliminated. Additional constituents can also be added.
2. Analytical technique. Because procedures are constantly evolving, specific procedures must be defined, as well as detection limits and quality assurance standards. This may be the single most important interlaboratory variable in this bioassessment technique.

#### Interpretation of Results

Results of bioassay and bioaccumulation studies are interpreted by statistical techniques recommended by the U.S. EPA and COE (1977). When warranted, each data set is evaluated by Cochran's test to determine if variances are homogeneous. If homogeneous variances occur, a parametric, unpaired "t" test or parametric one-way analysis of variance (ANOVA), and, if necessary, Student-Newman-Keul's test are used to determine the presence or absence of statistically significant differences. If variances are not homogeneous, data is transformed (natural logarithm of X+1) and analyzed as described above. Transformed data exhibiting heteroscedasticity are analyzed using approximate, unpaired "t" tests or nonparametric ANOVAs and, if necessary, Wilcoxon-Mann-Whitney's STP test. If greater than 50 percent mortality occurs at any concentration of liquid or suspended

particulate phase during bioassays, the environmental concentration of liquid or suspended particulate phase after the 4-hour period of initial mixing is estimated by the release zone method, LC50 values are calculated and an exposure-time-dependent limiting permissible concentration (LPC) is calculated and graphically compared to the estimated environmental concentration (dilution curve) of the phase.

APPENDIX C: INDIVIDUAL TIERED TESTING PROGRAMS DEVELOPED BY EACH  
TECHNICAL PARTICIPANT. INPUT FROM EACH PARTICIPANT HAS BEEN  
FAITHFULLY REPRODUCED IN ITS ENTIRETY EXACTLY AS SUBMITTED  
BY THE INDIVIDUAL

Dr. Bill Adams  
Monsanto Chemical Company  
St. Louis, Mo.

Bill Adams ☺

First, some comments. Of all the tests we have discussed I consider only the following to be acceptable for use at the present time on a routine basis.

1. Acute lethality tests
2. Life cycle tests
3. Bioaccumulation tests  
and possibly
4. Freshwater microcosm tests

From the view of a regulator (and industry), test methods have to be quantitative, standardized, routinely performed or be able to be performed routinely by many laboratories. They must incorporate quality assurance and quality control measures and the variability and predictiveness of the test must be understood. There aren't many sediment tests which meet these criteria, but there are a few which come close. (Sister Chromatid)

I reject the anies test and the adenyate energy test<sup>1</sup> because they are not highly reliable nor do we know how to use the results. Bioenergetic and trophic transfer tests are expensive, but could be used in special cases. Histopathology is very expensive, and techniques are just now emerging. It is too soon to use this as a routine tool for sediment assessment.

My approach is to keep things simple and to use easily performed, routine tests which are sensitive indicators of toxicity and bioaccumulation.

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Tier I

acute lethality test (midge, amphipod, <sup>use 1 or more</sup> daphnid, fish)

- Failure of test ends all disposal in water and requires no more testing
- Passing (ie  $\geq 80\%$  survival) requires Tier II

Tier II

1. Part life cycle test or full life cycle test (midge, amphipods, xenopus <sup>frog</sup> eggs, Ceriodaphnia or daphnids could be used)

- At least 2 species should be tested
- Failure to pass ends open water disposal and requires no more testing
- Passing requires a bioaccumulation study before open disposal is allowed

2. Bioaccumulation (use midge, amphipods, bottom feeding fish)

- A measurement of bioaccumulation could be obtained during the life cycle test, by measuring the organisms chemical levels at the end of the test. This measurement would not be needed if the toxicity portion of the test is failed.

- Accumulation of significant residues ie a) in excess of FDA action levels  
b) in excess of concentrations known to cause effects on aquatic organisms  
c) or in excess of some consensus safe level results in failure of the bioaccumulation test

3. Bulk chemistry measurements I placed these 3rd because of their cost. Concentrations of certain chemicals in excess of state standards would be cause for rejection of open water disposal.

## Tier III

Specialized tests such as microcosms, trophic transfer or bioenergetics could be used on a case by case examination. Lack of confidence or ambiguity in data from previous tiers.

## Comment

Use of a sensitive life stage test early in the testing program should be cost effective, by quickly eliminating those sediments which are unacceptable for open water disposal.

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Dr. Ray Alden  
Old Dominion University  
Applied Marine Research Laboratory  
Norfolk, Va.

Ray Allen

- Tier I: 1) Bulk sediment analysis + evaluation to determine potential problems (eg. beach sand vs. sediments w/ PCBs)  
2) Evaluation of bioaccumulation potential (when work on metals have been completed)
- Tier II: 1) Ames Test screening (when methods become available)  
2) Acute bioassays on sensitive yeast species (solid phase) - If sublethal tests can be run, included concurrently w/ little additional resources they should be
- Tier III: 1) Acute lethality + bioaccumulation - (on additional species)

Tier IV:

- 1) Trophic transfer (on those sediments making questionable bioaccumulation results)
- 2) Life cycle (or partial life cycle) tests

- Tier V:
- 1) microcosms (or mixed species bioassays)
  - 2) bioassays
  - 3) ~~single chemical testing~~
  - 4) AEC
- (unless techniques become routine, rapid, & widely available for screening large #'s of samples in an earlier tier)

Mr. Jim Bajek  
US Army Engineer Division, New England  
Regulatory Branch  
Waltham, Mass.

Workshop Participant: Jim Bajek 18 April 85

Below is a list of subjects, including bioassessment techniques, which were discussed during the workshop. Please arrange the items in a tiered testing hierarchy for a regulatory testing program. Items appearing within each tier should be prioritized numerically. This is your opportunity to provide specific input to the workshop. Comments are strongly encouraged regarding your rationale for the tier, on the individual items and on any aspect of the workshop.

Thank you for your input.

\* See Note on reverse

- ②b Acute Lethality Tests - use <sup>at least 3 types of organisms representative of disposal area. Use density + suspension feeders</sup> <sup>use tied in with commercial, recreational + human health aspects</sup> ⑥ Histopathology - do this only for special "problem" cases
- ⑦ Adenylate Energy Charge - seems <sup>to need more research</sup> ②a Life Cycle Tests - This should be done to develop protocol for 2b.
- ④ Ames Test - may have good utility as a quick screen for bioassay substrates before bioassay analyses are performed ⑨ Microcosms - special problem projects only
- ③ Bioaccumulation - only for constituents of major concern ⑩ Sister Chromatid Exchange - needs more research
- ⑧ Bioenergetics - This is more of a research item, the information which should be used to evaluate all of the other tests ⑤ Trophic Transfer - This should be used to evaluate the bioaccum. results when uptake is found to occur.
- ① Other Bioassessment Tests  
① bulk sediment tests (physical + chemical) - use for gross preliminary screening + dredge/disposal management

Note \* These bioassay testing programs should be considered / used only after other basic project related information is developed to determine the actual need for bioassay testing. This additional information includes a review of existing information relative to the proposed dredge area (i.e., physical, chemical + biological test data, dredging history - frequency + amount of material, possible contaminant sources (point + nonpoint) + any scientific data that could be used to evaluate the potential impact of dredging + disposal of the material at the expected locations. If all available information can be used to sufficiently predict the impacts then no further testing should be required. Otherwise, the tiered testing protocol should be followed.

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Dr. Gary Chapman  
US Environmental Protection Agency  
Environmental Research Laboratory  
Corvallis, Oreg.

Workshop Participant: Gary Chapman 18 April 85

Below is a list of subjects, including bioassessment techniques, which were discussed during the workshop. Please arrange the items in a tiered testing hierarchy for a regulatory testing program. Items appearing within each tier should be prioritized numerically. This is your opportunity to provide specific input to the workshop. Comments are strongly encouraged regarding your rationale for the tier, on the individual items and on any aspect of the workshop.

Thank you for your input.

---

Acute Lethality Tests - 5 - Histopathology  
Adenylate Energy Charge - 2 in one test - 2 - Life Cycle Tests  
4 Ames Test 7 - Microcosms  
1 Bioaccumulation (by route from chemistry) Sister Chromatid Exchange  
Bioenergetics 6 - Trophic Transfer  
Other Bioassessment Tests

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Gary Chapman

1. If models of sed. to fish tissue are acceptable to the regulating agency, start with such chemistry (and normalizing factors) for those chemicals with regulated tissue values (eg. PCB, DDT-DDE, dieldrin). IF sed. fail the screen. STOP if they pass go to 2.

2. Conduct acute chronic toxicity of solid phase (as described by Bill Kelams) using a cladoceran (Daphnia magna, Daphnia pulex) and a midge or amphipod, or perhaps also to a fish 10 day. Look at mortality, growth & reproduction. If sed. fail stop. If they pass go to 3. Check on tissue levels as check for step 1. (if desired)

3. Conduct an acute bioassay IF you think you can interpret them in a rational manner. This type of test might not be a good bioassessment technique.

Alternatively go to a longer exposure test (eg. the medaka)

Alternatively assume (pragmatically, if not necessarily rationally) that a bioassessment

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in excess of "background" levels & had  
on a non-degradation basis. This is hard  
to defend on a cause-effect basis, but  
not on a conservative pragmatics basis.

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have the right to reproduce

Mr. Richard Krauser  
US Army Engineer District, New York  
Water Quality Compliance Section  
New York N.Y.

Workshop Participant: Richard Krueger 18 April 85

Below is a list of subjects, including bioassessment techniques, which were discussed during the workshop. Please arrange the items in a tiered testing hierarchy for a regulatory testing program. Items appearing within each tier should be prioritized numerically. This is your opportunity to provide specific input to the workshop. Comments are strongly encouraged regarding your rationale for the tier, on the individual items and on any aspect of the workshop.

Thank you for your input.

Lower numbers = tier; circled numbers = priority within tier

I(1) Acute Lethality Tests

x Histopathology

Adenylate Energy Charge

II(2) Life Cycle Tests

I(3) Ames Test

III(1) Microcosms

II(1) Bioaccumulation

x Sister Chromatid

Bioenergetics

Exchange  
Trophic Transfer

Other Bioassessment Tests

I(2) Thymidine incorporation  
III(2) Recolonization

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1st Phase

I. First tier:

A. Screen test - A short, rapid, preliminary test, to be done in one session.

B. Thermodynamic calculations of maximum body burden - would screen out candidates for bioaccumulation test.

C. Acute toxicity - address synergistic effects; solid phase only which would address long term impacts.

II. Second tier:

A. Bioaccumulation - constituents tested would be those red-flagged by thermodynamic calculations and are known to persist. Baseline studies should be conducted to serve as reference for no further degradation or whatever policy is decided.

III. Third tier:

A. Thermostom - to address toxicity or total community.

B. Life cycle testing - to measure growth as a measure of potential reproductive success.

C. Recolonization - to address community changes and degradation of total community.

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Mr. Michael Mac  
US Fish and Wildlife Service  
Great Lakes Fisheries Laboratory  
Ann Arbor, Mich.

Workshop Participant: Michael Mac 18 April 85

Below is a list of subjects, including bioassessment techniques, which were discussed during the workshop. Please arrange the items in a tiered testing hierarchy for a regulatory testing program. Items appearing within each tier should be prioritized numerically. This is your opportunity to provide specific input to the workshop. Comments are strongly encouraged regarding your rationale for the tier, on the individual items and on any aspect of the workshop.

Thank you for your input.

---

Acute Lethality Tests  
Adenylate Energy Charge  
Ames Test  
Bioaccumulation  
Bioenergetics

Histopathology  
Life Cycle Tests  
Microcosms  
Sister Chromatid  
Exchange  
Trophic Transfer

Other Bioassessment Tests

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FIRST LEVEL  
SCREEN

MCC  
HISTORICAL EVALUATION OF POTENTIAL CONTAM  
BULK CHEMICAL ANALYSIS, TOL, O/G  
PHYSICAL COMPOSITION

POSSIBLE CONTAMINATION

CLEAN-  
UNCONTROLLED  
DISPOSAL

SECOND LEVEL - ① ACUTE TOXICITY -

SERRIOAPHNA OR DAPHNA - WHOLE SEDIMENT  
AND LARVAL FATHEAD 48-96 h STATIC

② CHRONIC TOXICITY & REPRODUCTION -

CHIRONOMID OR SURVIVAL, GROWTH, REPRODUCTION  
WHOLE SEDIMENT

PONTOPOREIA  
(PENDING METHODOLOGY  
DEVELOPMENT)

③ BIOACCUMULATION -

FATHEAD 10 DAY LOW FLOW-THROUGH  
& WHOLE SEDIMENT  
EARTHWORM (OR OTHER DEPOSIT FEEDER)  
NEED NOT BE RESUSPENDED.

④ CARCINOGENICITY - MUTAGENICITY

AMES TEST - IF RESEARCH SHOWS APPLICABILITY  
OR TO ACTUAL FISH TUMOR INDUCTION

MEDAKA - WHOLE SEDIMENT EXPOSURE.

CERT ... does not  
permit full ... reproduction

FOR THE MOST PART AVAILABLE METHODOLOGIES  
WILL PROVE SUFFICIENT. I WOULD LIKE TO  
SEE DEVELOPMENT OF PONTOPOREIA AS A  
POSSIBLE CHRONIC TEST SUBJECT.

THE ONLY AREA IN NEED OF EXTENSIVE  
RESEARCH IS THE CARCINOGENICITY TEST.  
THE AMES TEST WILL STREAMLINE THIS  
BIOASSAY BUT IT NEEDS TO BE DEMONSTRATED  
THAT IT IS RESPONSIVE AND APPLICABLE TO  
FISH TUMOR INDUCTION FROM AN ACTUAL  
SEDIMENT EXPOSURE.

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Dr. Joe M. O'Connor  
New York University Medical Center  
Tuxedo, N.Y.

O'Connor

TIER I. General tests and screening

1. Limited Bulk sediment analysis
2. HPTLC/Ames assay
  - a. mutagens
  - b. identify toxic/mutagenic compound classes
3. Calculate equilibrium BCF factors

TIER II. Specific Toxicity testing

1. Acute lethality
  - a. Possible coordination with studies of burden/effect relationships
    - a. histopathology
    - b. SCE

#

TIER III. Chronic Toxicity / Sublethal Effects

1. Combine Bioaccumulation / Trophic Transfer (microcosms?)
2. Life cycle testing
3. Microcosms combined with bioaccumulation (if possible)

O'Connor

## TIER IV Physiological / Biochemical

1. Enzyme induction (e.g. AHH enzymes)
2. Tumorigenicity / carcinogenicity
3. Various physiological end-point assays
  - a. Respiration
  - b. Homeostasis
  - c. Scope for growth

## Conceptual Basis for Tiers

I. Identify what you have, what possible human health effects might be, and what maximum equilibrium BCF may be for critical metallic/organic compounds

Establish flags for go/no go decision

II. Tier II aimed at finding out if you kill or maim with test material. One should not lose the opportunity to determine burden/effects data by applying analysis to dead matter

III. If material must go to III, then the best approach is the multi-species bioaccumulation study combined with trophic transfer. Further information can be gained here on concentration effects, and to verify calculated equilibrium BCF from Tier I.

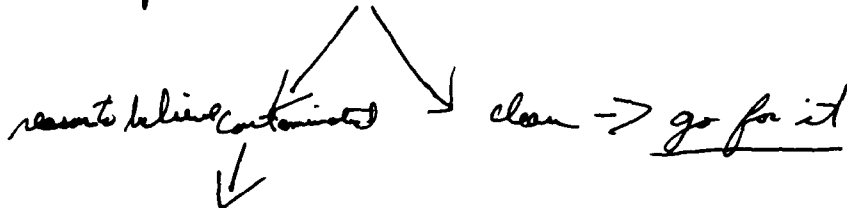
IV. Tiers II & III can really go any way. The critical thing is to apply physiologically biochemical tests most meaningful to the problem at hand.

Tom: Good work; but one yet (that's what I mean). I think we did our job, and I think the Wisconsin people know it!

Mr. Norman I. Rubinstein  
US Environmental Protection Agency  
Environmental Research Laboratory  
Narragansett, R.I.

# Non-Resubmit

Tier I. Determine on the basis of ~~source~~ source inputs and historical use ~~the~~ the degree of potential contamination.



Tier II. A. Bulk Sed Analysis  
B. Predict bioavail Concentration. (Model)  
(B = Exposure Concentration)

Exposure Concentration  
are  
of concern

actual Exposure concentration below  
limits of concern  
→ open beyond

Tier III. A. Acute lethality tests.  
B. Ames test.  
C. Bioaccumulation tests. } Single Bioassays  
end points must be evaluated within a management strategy

i.e. A. → any toxicity ( $\geq 10\%$ ) is grounds for permit denial  
B. Mutagenicity → ?  
C. Bioaccumulation → any degradation vs. further spread?

Tier III end points must be interpreted <sup>along</sup>  
with ~~the~~ Socio-economic ~~features~~ <sup>inputs</sup>.

This will either force the issue to further  
Testing. I.R. <sup>Tier IV</sup> → High Cycle, Troop Transfer  
History path ect. and others

that apply.  
or resolve the Design question.

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Dr. John Scott  
Science Applications International Corporation  
Newport, R.I.



2000001  
Tier 1. In addition to the above to include some  
some knowledge of the potential of potential  
mistake.

The Ames + microtox although not widely used  
for sediment testing do show promise because of  
the ease of developing a large database.

Tier 2 here we return to the old standards,  
bioassay and bioaccumulation - the selection  
of test species is most important the  
Dredge, some fish, and an amphipod would  
show the most, however, for bioacc.

probably need a fish and large worm.

- bioassay listed here because it can act as  
an early warning system e.g. if quantified  
and could act in Mac's job standard  
story.

Tier 3 Should we go to this tier life  
cycle testing looking at growth would  
show the most promise - to carry  
bioaccum one step further - some  
trophic transfer studies would be  
helpful - the Bioenergetics, PEC,  
SCE would fall in this category  
but with not much utility in this  
case.

Tier 4 These last two Regol. + Microcosm  
maybe the closest thing to regulatory  
control but only in the most extreme  
cases because of cost

~~SECRET~~  
314 ED AMP-  
(TUMOR) AND/OR - 2001 - 11,

TIER 1  
① Ames / ON TLC  
② MICROTOX

TIER 2  
① Lethality Acute  
② Bioaccumulation  
③ HISTOLOGY (pre-tumor)

TIER 3  
① LIFE CYCLE (GROWTH)  
② TROPHIC TRANSFER  
③ Bioenergetics  
④ AEC, SLE

TIER 4  
① RECOLONIZATION  
② MICROCOSMS

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Mr. Tim Ward  
Erco: A Division of Ensaco, Inc.  
Cambridge, Mass.

# Tier 1, 2, 3

Tier 1 1. Acute Lethality Tests

Tier 2 1. Bioaccumulation  
2. Ames  
3. Life cycle tests (partial chronic tests also)

Tier 3 1. Microcosm 5. Trophic transfer  
2. AEC 6. Bioenergetics  
3. Histopathology  
4. Sister Chromatid Exchange

Tier 1. Tests are simple, cost effective and have well defined endpoints. Their use is limited to screening only, and as such they will have a very limited usefulness in predicting field effects.

Tier 2. Increasingly powerful predictors of field effect, however each has a number of questions associated with the meaning of the endpoints. The advantages are that methods have been relatively well defined, a data base exists, and they can be tailored to answer important, specific questions.

Tier 3. With the exception of microcosm tests, these tests have more use in attempts to understand cause and effect rather than predicting effects. Open water disposal. While most provide additional important data, their complexity makes them inappropriate for earlier tiers. Microcosm tests are an ideal last step prior to disposal.

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APPENDIX D: WORKSHOP EVALUATIONS. INPUT FROM WORKSHOP  
MODERATOR NOT INCLUDED

Technical Participant

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

5 4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 4 3 2 1

*the format & time available was real good.*

4. The objectives of the workshop were met.

5 4 3 2 1

*← some things were left out*

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 4 3 2 1

*a good experience for me from a technical & research point of view*

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 4 3 2 1

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Technical Participant

WORKSHOP EVALUATION

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1. The purpose and objectives of the workshop were clearly stated.

(5) 4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 (1)

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

(5) 4 3 2 1

4. The objectives of the workshop were met.

5 (4) 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

(5) 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

(8) 7 6 5 4 3 2 1

Technical Participant

WORKSHOP EVALUATION

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5 (4) 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 (1)

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 (4) 3 2 1

4. The objectives of the workshop were met.

(5) 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 3 2 1 No comment

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of ~~food, drink, and~~ local color.)

(5) 4 3 2 1



Technical Participant

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

5 (4) 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 (2) 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

(5) 4 3 2 1

4. The objectives of the workshop were met.

5 (4) 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 (4) 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 (4) 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 (4) 3 2 1

Technical Participant

WORKSHOP EVALUATION

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5 (4) 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 (2) 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

(5) 4 3 2 1

4. The objectives of the workshop were met. *Depends on your report.*

5 (4) 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

(5) 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.) *To. Caloric*

5 (4) 3 2 1

Technical Participant

WORKSHOP EVALUATION

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2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 (2) 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

(5) 4 3 2 1

4. The objectives of the workshop were met.

(5) 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

(5) 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 (4) 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 4 (3) 2 1

Technical Participant

WORKSHOP EVALUATION

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5 (4) 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 (1)

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 (4) 3 2 1

4. The objectives of the workshop were met.

5 (4) 3 2 1

*I can't honestly put a 5 until I see the written report but I'm encouraged.*

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 (3) 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

(5) 4 3 2 1

*My only disappointment was I bit of overkill on marine methodology when more freshwater information could have been presented in the initial short presentation.*

Technical Participant

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

(5) 4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review. *But the conduction of a literature review wouldn't hurt.*

5 4 3 2 (1)

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

(5) 4 3 2 1

4. The objectives of the workshop were met.

(5) 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour). *One hr for lunch was all that was needed. The "no breaks" concept worked very well.*

5 4 (3) 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

(5) 4 3 2 1

Technical Participant

WORKSHOP EVALUATION

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2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 4 3 2 1

4. The objectives of the workshop were met.

5 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 4 3 2 1

Technical Participant

WORKSHOP EVALUATION

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1. The purpose and objectives of the workshop were clearly stated.

(5) 4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 (2) 1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 (4) 3 2 1

4. The objectives of the workshop were met.

(5) 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 (3) 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

(5) 4 3 2 1

Observer

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

5  4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2  1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5  4 3 2 1

4. The objectives of the workshop were met.

5 4  3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 3 2 1 No opinion

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 4 3 2 1 No opinion

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 4 3 2 1



Observer

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.  
⑤ 4 3 2 1 I'm NOT SURE PARTICIPANTS WERE WELL AWARE OF OBJECTIVES ON ARRIVAL, BUT ALL SEEMED SO AFTER 1<sup>ST</sup> SESSION.
2. The objectives could have as easily been met by conducting a thorough literature review.  
5 4 3 ② 1 I AM SOULDLY CONVINCED THE FORMAT WAS THE BEST POSSIBLE FOR THE NEED.
3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.  
⑤ 4 3 2 1 THE COMFORTABLE, OPEN INTERCHANGE CAUSED BY THE DYNAMICS OF THE STRUCTURE
4. The objectives of the workshop were met. WAS VERY REVEALING OF INDIVIDUAL INPUTS AND EMERGING CONSENSUS OR DIFFERENCES AS APPROPRIATE,  
⑤ 4 3 2 1
5. No formal morning or afternoon breaks, but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).  
⑤ 4 3 2 1 IT PROVIDED FOR A GOOD CONTINUUM.
6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)  
⑤ 4 3 2 1 I AM PLEASED TO HAVE BEEN PRESENT. I COULD NOT CONCEIVE OF A BETTER MANNER FOR THIS RESEARCH.
7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)  
⑤ 4 3 2 1

Observer

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

5 (4) 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2 (1)

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5 (4) 3 2 1

4. The objectives of the workshop were met.

(5) 4 3 2 1

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 (4) 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

(5) 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

(5) 4 3 2 1

Observer

WORKSHOP EVALUATION

Please answer the statements below by circling one number between 1 and 5 where 5 equates to a complete "Yes" and 1 to a complete "No". Comments on all of the items below are strongly encouraged and would be appreciated.

1. The purpose and objectives of the workshop were clearly stated.

5  4 3 2 1

2. The objectives could have as easily been met by conducting a thorough literature review.

5 4 3 2  1

3. The structure of the workshop, (i.e., initial short presentation followed by round table discussion), was the best format to achieve the objectives.

5  4 3 2 1

4. The objectives of the workshop were met.

5  4 3 2 1 AT LEAST FOR THE "ORAL" PART

5. No formal morning or afternoon breaks but an extended lunch (1-1/2 hours) was more desirable than having the two breaks and a shorter lunch period (1 hour).

5 4 3 2 1

6. The workshop was worth the trip to Milwaukee. (Please be honest and disregard the influence of food, drink, and local color.)

5 4 3 2 1

7. The workshop was worth the trip to Milwaukee. (Please be honest and include the influence of food, drink, and local color.)

5 4 3 2 1

APPENDIX E: FORMAL COMMENTS FROM WORKSHOP PARTICIPANTS ON  
DRAFT PROCEEDINGS.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL RESEARCH LABORATORY - DULUTH  
6201 CONGDON BOULEVARD  
DULUTH, MINNESOTA 55804

Gary Chapman  
Western Fish Toxicology Station  
1350 S E Goodnight Avenue  
Corvallis, OR 97330

August 13, 1985

John R. Sullivan  
Surface Water Stds & Monitoring  
Wisconsin DNR  
Box 7921  
Madison, WI 53707

Dear John:

I have made some editorial changes in my closing comments at the workshop. I trust you'll allow that, because these changes certainly clarify my brief input.

I've no problem with the overall report. Few people will want to read much beyond the summary which has been sneakily buried on pages 47-49. I fear that some readers may never find it.

I'd like to thank you all for the chance to participate in the workshop. In case you hadn't heard, we're officially scheduled to move to Duluth on-or-before July 30, 1986. It's getting closer, but I've still got several straws I'm grasping at.

Best wishes,

A handwritten signature in cursive script that reads "Gary".

Gary A. Chapman  
Research Aquatic Biologist

"1) Bioaccumulation

2) Acute Lethality Tests and 3) Life Cycle Tests, in one test.

4) Ames Test

5) Histopathology

6) Trophic Transfer

7) Microcosms:

1. If model of <sup>transfer of chemicals</sup> sed<sup>iment</sup> to fish tissue are acceptable to the regulatory agency, start with bulk chemistry (and normalizing factors) for those chemicals with regulated tissue values ( eg. PCB, DDT-DDE, dieldrin). If sed<sup>iments</sup> fail the screen, Stop. If they <sup>pass</sup> fail go to 2.

2. Conduct acute-chronic bioassay of solid phase (as described by Bill Adams) using a cladoceran (Ceriodaphnia, Daphnia pulex) and a midge, or amphipod. Perhaps also do a fish. 10 day. Look at mortality, growth & reproduction. If sed<sup>iments</sup> fail, Stop. If they pass go to 3. Check on tissue levels as check for step 1; (if desired).

3. Conduct an Ames type test if you think you can interpret <sup>results</sup> them in a rational manner. If you fail all sed<sup>iments</sup>, this might not be a good bioassessment technique.

Alternatively go to a longer oncogenicity test (eg. the Medaka.)

Alternatively assume (pragmatically, if not necessarily rationally) that a bioconcentration in excess of "background" levels is bad on a nondegradation basis. This is hard to defend on a cause-effect basis, ~~but not on a conservative pragmatic basis.~~

**ROUTING AND TRANSMITTAL SLIP**

Date *July 31, 1985*

TO: (Name, office symbol, room number, building, Agency/Post)		Initials	Date
1.	<i>John Sullivan</i>		
2.			
3.			
4.			
5.			

AUG - 1985

Action	File	Note and Return
Approval	For Clearance	Per Conversation
As Requested	For Correction	Prepare Reply
Circulate	For Your Information	See Me
Comment	Investigate	Signature
Coordination	Justify	

**REMARKS**

*These are my only comments.  
I use the term "red-fogged" to indicate  
those constituents which have been shown,  
by thermodynamic calculations, to be  
cause for concern*

DO NOT use this form as a RECORD of approvals, concurrences, disposals, clearances, and similar actions

FROM: (Name, org. symbol, Agency/Post) *John Sullivan* Room No.—Bldg. \_\_\_\_\_  
*John Sullivan* Phone No. \_\_\_\_\_  
*John Sullivan* (212) 264-5621

5041-102

☆ U.S. G.P.O. 1982-387-346

OPTIONAL FORM 41 (Rev. 7-78)  
 Prescribed by GSA  
 FPMR (41 CFR) 101-11.206

Copy over to DTIC does not  
 permit full legible reproduction

*Rich Kruss*

"Roman numeral = tier; encircled numbers = priority within tier

I (1) Acute Lethality Tests

I (2) Thermodynamic calc.

I (3) Ames Test

II (1) Bioaccumulation

II (2) Life Cycle Tests

III (1) Microcosms

I. First tier

A. Ames Test- to address potential mutagenicity; will provide a worst case situation.

B. Thermodynamic calculations of maximum body burden- would screen out constituents for bioaccumulation test.

C. Acute lethality bioassay- addresses synergistic effects; solid phase only which would address long term impacts.

II. Second tier

A. Bioaccumulation- constituents tested would be those ~~red flagged~~ <sup>red-flagged</sup> by thermodynamic calculations and are known toxicants. Baseline studies should be

calculated to serve as reference for no further degradation or whatever policy is decided.

III. Third tier

A. Microcosms- to address toxicity on total communities.

B. Life cycle testing- specifically growth as a measure of potential reproductive success.

C. Recolonization- to address community changes and degradation of total community."





State of Wisconsin

DEPARTMENT OF NATURAL RESOURCES

Carroll D. Besadny  
Secretary

BOX 7921  
MADISON, WISCONSIN 53707

File Ref: 3200

July 12, 1985

Mr. Richard Krauser  
U.S. Army Engineer District, New York  
Water Quality Compliance Section  
26 Federal Plaza  
New York, New York 10278

Dear Mr. Krauser:

Enclosed is a copy of the draft report entitled, "Bioassessment Methodologies for the Regulatory Testing of Freshwater Dredged Material." As a workshop participant you have played a key role to this point; however, additional effort on your part is necessary to ensure the accuracy of the proceedings.

Please review your comments as summarized in the proceedings and your pre-workshop submittals in detail and the rest of the document in general. Please send me your comments no later than August 16, 1985. We are intending to append the comments to the proceedings.

A recommendation to establish a tiered testing approach for dredge material evaluation has been given to agency administration. Hopefully, in the near future the State of Wisconsin will adopt such a strategy.

Once again, thank you for your attendance at the workshop and your comments on the proceedings.

Sincerely,  
Bureau of Water Resources Management

  
John R. Sullivan  
Surface Water Standards & Monitoring Section

JRS:jms  
Enc.



## United States Department of the Interior

FISH AND WILDLIFE SERVICE  
Great Lakes Fishery Laboratory  
1451 Green Road  
Ann Arbor, Michigan 48105

IN REPLY REFER TO:

August 15, 1985

Dr. John R. Sullivan  
Bureau of Water Resource Management  
Wisconsin Department of Natural Resources  
Box 7921  
Madison, Wisconsin 53707

AUG 15 1985

Dear John:

I would like to compliment Tom Dillon and Alfreda Gibson on the excellent job of presenting the workshop proceedings. The draft reads well, seems mostly accurate and contains useful information. My only concern is the section on temperature on page 24. Perhaps it may have been taken somewhat out of context, and thus seems incomplete but I don't recall making the statement that "...only true bottom temperatures should be considered." Surely bottom temperatures would be environmentally realistic for evaluating sediments at a disposal site, given that 1) sediments do not migrate out of this area to somewhere that elevated temperatures may occur or 2) sediment associated contaminants will not migrate out of the area through processes associated with sediment/water interface. Also, depending on the testing procedure that the state adopts, water temperature at the dredging sight may want to be considered.

The influence of water temperature on acute toxicity is not well understood and appears to be compound specific. Acute testing at standardized temperatures may be more comparable to existing toxicity data and may also allow for best survival of control organisms. I'm not sure all this information needs to be put into the report but at least I would suggest changing line seven to: "...and that true bottom temperatures should be considered for evaluating acute toxicity at a disposal site." Also, line four of that paragraph would be more accurate to read "...May through September."

Again, I laud the efforts of Tom and Alfreda and also of your office for sponsoring this endeavor. If I can be of further assistance, please call (313-994-3331).

Sincerely,

Michael Mac  
Fishery Research Biologist

**ERCO**

205 Alewife Brook Parkway, Cambridge, Massachusetts 02138 (617) 661-3111 Telex 650-256-7697 (MCI)

A DIVISION OF

**ENSECO**  
INCORPORATED

AUG 12 1985

August 7, 1985

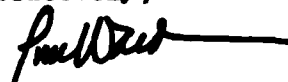
John R. Sullivan  
Surface Water Standards and  
Monitoring Section  
State of Wisconsin Department  
of Natural Resources  
Box 7921  
Madison, Wisconsin 53707

Dear Mr. Sullivan:

I have reviewed the draft report from the Workshop on Bioassessment Methodologies for the Regulatory Testing of Freshwater Dredged Material. The text accurately describes the discussion and conclusions of the workshop, and I cannot add anything of value to the document.

I thoroughly enjoyed the workshop and found the discussions to be thought-provoking and meaningful. Please let me know if I can be of any further assistance.

Sincerely,



Timothy J. Ward  
Director, Aquatic  
Toxicology Laboratory

TJW:sbm

Regional and international offices:

- Suite 115, Statesman Insurance Building, 3815 Montrose, Houston, Texas 77006 (713) 523-7311
- 525 Central Avenue, Cedarhurst, New York 11516 (516) 295-1162
- c/o Bectech Trading Co., Ltd., P.O. Box 101-41, Taipei, Taiwan (R.O.C.) Tel. 5013908

**SAIC**  
JRB Associates  
Marine Services Branch

September 10, 1985

Mr. John R. Sullivan  
Surface Water Standards & Monitoring Section  
Department of Natural Resources  
Box 7921  
Madison, WI 53707

Dear John:

I have reviewed the report on "Bioassessment Methodologies" and my general comments about its accuracy are that the report fairly will reflect the concensus or lack thereof among the group of participants. There are a few textual errors which are listed on the enclosed sheet.

The only other comment I have regards the discussion, beginning on page 15, of hazard assessment. It seems we have taken the tiered testing approach out of the more general context of hazard assessment where site and waste characterization and monitoring are integral components. (see enclosed figure). The report describes some site and waste characterization as part of the initial evaluations in tiered testing. The monitoring component evaluates the accuracy of risk predictions and allows for further regulatory input, albiet, after a disposal decision has been made. This is not a major part, but maybe figure 1 on page 15 could be revised.

I've also enclosed an internal EPA planning document on tiered testing for your review. If you have any questions please call me at (401) 789-1071.

Sincerely,



K. John Scott  
Senior Scientist

KJS:kl

Enclosure

*JRB Associates, a Company of Science Applications International Corporation  
c/o EPA, South Ferry Road, Narragansett, RI 02882*

SPECIFIC COMMENTS

- P. 16-17: Discussion of the Thermodynamic model as associated with bulk sediment analyses should note that the model is under development.
- P. 27: 4th line of paragraph 2, change body length and weight to survival.
- P. 29: 1st line paragraph 1 should read: Dr. Scott briefly explained bioenergetic endpoints being investigated at the EPA-Narragansett....
- P. 29: Last paragraph, first line, strike behavioral, also, the heading of this section is inappropriate, they are not all behavior.
- P. 30 Last paragraph, 3rd sentence should read: Small but significantly different SCE rates have been observed in....

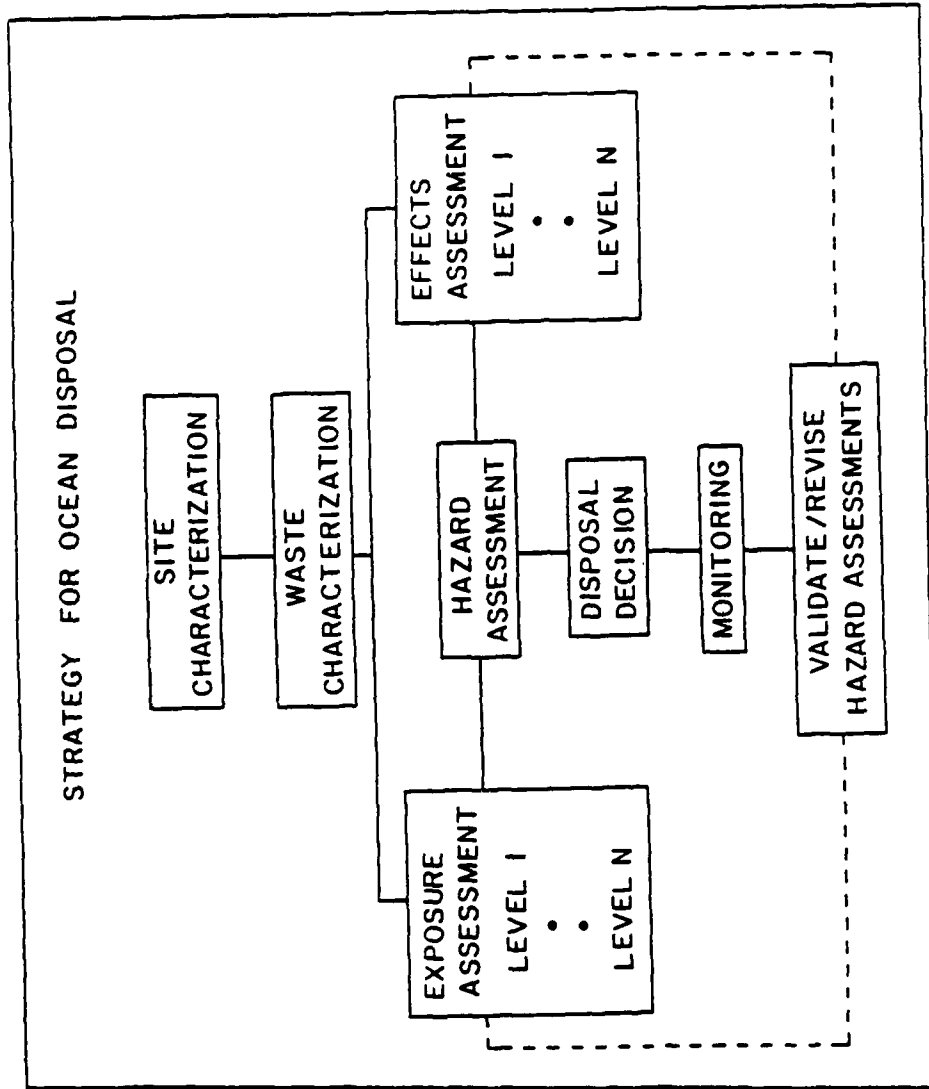


Figure 1. Ocean disposal research strategy showing the interrelationships between the individual components, the tiered structure for the effects and exposure assessment elements.

**SAIC**  
**JRB Associates**  
Marine Services Branch

June 11, 1985

1985

Dr. John Sullivan  
Wisconsin Department of Natural Resources  
Box 7921  
Madison, WI 53707

Dear Jack,

Sorry I've been so late getting this expense report to you, it seems I've been swamped since returning from Wisconsin. Thanks again for putting on such a good workshop. I might add that Tommy, Norm Rubinstein and myself have recently become involved with many others in the rewrite of the so called "Green Book" and the tiered testing approach for marine waters. Our initial discussions were very similar to the conclusions reached in your workshop, and we've found it very helpful in that respect.

Also, enclosed, you'll find a draft of the overview section of our DAMOS annual report and symposium held last winter. To briefly summarize the program, DAMOS has developed monitoring tools which can aid the manager ( in this case the COE New England Division) in decision making regarding site designation, dredged material disposal control and subsequent monitoring. Our capabilities cover the range of physical, chemical and biological sampling, analysis and interpretation. This year we are beginning to develop a tiered monitoring approach, outlined in the enclosed table, and a decision making framework (flow chart) within which to apply these methods. We feel that this framework will provide a more cost effective approach to monitoring than has been the case over the last several years. Should open water disposal in Wisconsin become a reality in the near future, some of these techniques may be useful to your program.

Again, thanks very much for your hospitality, and please call me if you have any questions about the DAMOS program or SAIC capabilities.

Sincerely,



K. John Scott  
Senior Scientist

KJS: ek

Enclosure

*JRB Associates, a Company of Science Applications International Corporation*  
c/o EPA, South Ferry Road, Narragansett, RI 02882



DEPARTMENT OF THE ARMY  
NEW ENGLAND DIVISION, CORPS OF ENGINEERS  
424 TRAPELO ROAD  
WALTHAM, MASSACHUSETTS 02254

Aug 30 1985

REPLY TO  
ATTENTION OF

August 27, 1985

NEDOD-R  
Regulatory Branch

Mr. John R. Sullivan  
Surface Water Standards and Monitoring Section  
Wisconsin Department of Natural Resources  
Box 7921  
Madison, Wisconsin 53707

Dear Mr. Sullivan:

I have reviewed the draft report entitled "Bioassessment Methodologies for the Regulatory Testing of Freshwater Dredged Material". Generally, the report is an accurate outline of our various discussions during the workshop. However, the text on pages 22 and 23 indicate some confusion regarding the meaning and use of control vs. reference sediment in aquatic bioassay testing. I believe this can be clarified by reviewing pages 13 and 14 of the draft "Dredged Material Testing Guidance for Ocean Disposal" I previously furnished and which is included in your report. The reference material doesn't need to be related, either physically or chemically to the test material. More important, the reference material should be similar to the disposal site bottom condition before any disposal had occurred there. Control material should be from a "clean" area and be of a grain size suitable to maintaining the test organisms in a healthy status. The controls check for influences other than from the test or reference materials and act as a quality assurance measure during the entire handling and testing process.

I am pleased to be of assistance in helping you determine the appropriate testing methodology for your region. Please call (617) 647-8213 if there are any questions regarding my comments.

Sincerely,

James J. Bajek  
Dredged Material  
Management Section  
Regulatory Branch  
Operations Division





Applied Marine Research Laboratory  
(804) 440-4692 • Norfolk, VA 23508-8512

July 23, 1985

JUL 26 1985

Mr. John R. Sullivan  
Surface Water Standards and Monitoring Section  
Department of Natural Resources  
State of Wisconsin  
Madison, WI 53707

Dear John:

I have reviewed my copy of the report entitled "Bioassessment Methodologies for the Regulatory Testing of Freshwater Dredged Material" which was based upon the workshop in Milwaukee last April. I am gratified that a comprehensive written report was compiled to document a workshop that I considered to be a very worthwhile experience. However, I would like to clarify a few points:

<u>Page</u>	<u>Topic</u>	<u>Comments</u>
19	Suspended Phase (SP) Bioassays	I do not necessarily want to appear to be a lone "hold-out" for suspended solid bioassays. Our experience with SP tests could be regionally unique since the major toxins of the Port of Hampton Roads are PNAH's which are associated with the fine silt/clay fraction. This fraction is often flushed out of solid phase tests during water replacements. Therefore, we have adopted the use of a modified SP test (using the 100% elutriate only) for screening simply because it worked (i.e. test species in SP tests displayed the greatest mortalities). On the whole, however, I would recommend the use of solid phase bioassays for most bioassessment programs. We have always used solid phase tests on multiple species (along with microcosms) to confirm the results of the SP screening tests.

*Old Dominion University is an affirmative action/equal opportunity institution.*

<u>Page</u>	<u>Topic</u>	<u>Comments</u>
25	Microcosms	<p>I believe that there have been several misinterpretations of our experimental design for microcosms:</p> <ol style="list-style-type: none"> <li>1. Rather than a "re-colonization" approach, the microcosm might be more accurately described as a community toxicity test. The benthic macroinvertebrate communities are introduced to all experimental and control chambers. The defaunated sediments (test or control materials) are "dumped" on half of the communities. These treatments represent communities being buried by "clean" (control) or potentially toxic (test) sediments at a dredged material disposal site. The other set of treatments are communities exposed to the water masses receiving the simulated "dumps," but which are not directly covered by the sediments. These treatments explore the effects of disposal operations on communities living on the periphery of a disposal site.</li> <li>2. With respect to the results of the microcosm experiments run to date, it isn't completely true that there are "no real differences in communities (exposed to) contaminated dredged material relative to clean reference sediment." While it is correct that few species are completely eliminated by exposure to toxic sediments, highly significant changes in community structure of the benthos and zooplankton have been observed. Perhaps the statement could be made that the effects are surprisingly less dramatic than one would expect from the results</li> </ol>

of parallel static bioassay experiments. However, the effects did seem to be correlated with the relative toxicity of the sediments (as determined by other chemical and biological tests) and may, in fact, represent the sort of "impacts" that may be expected in the field following disposal operations.

3. It can be speculated that the elevated accumulation of PNAH's (and metals) in clams exposed to contaminated sediments in the microcosms relative to those taken from static bioassays may be due to the fact that the more "natural" conditions (currents, food supplies, etc.) of the microcosms stimulate feeding/ respiratory activities in the bivalves, while those in the 10-gallon tanks "clam up" when covered with the contaminated sediments. The increased uptake rates of the test species would be associated with the higher levels of these activities.
4. The cost of running a microcosm can hardly be considered to be "minimal" since it is an extremely man-power intensive effort (i.e. collection of disposal site water, zooplankton and benthic communities, taxonomic identification and enumeration, multivariate statistical analyses, etc.). However, a very sizeable data set can be gathered in a single experiment. Therefore, the relative toxicity of the sediments to all of the major taxa indigenous to the disposal

Page

Topic

Comments

site can be determined for the same relative costs as a series of multiple species bioassays.

5. Only macroinvertebrates (epibenthic and infaunal) were observed in the microcosms. Therefore, the statement concerning "meiofauna," while possibly true, cannot be substantiated. A variety of mobile macroinvertebrates were statistically shown to exhibit the avoidance response. Therefore, this response could be used as an indicator of stress. However, some of the less mobile species (e.g. bivalves, certain annelids) were seen to decline in relative abundance when exposed to contaminated sediments.
6. While natural seasonal changes in the community structure of indigenous fauna make the results of microcosms far from being directly reproducible, there is some evidence to indicate that conclusions may be the same when highly contaminated sediments are tested over various seasons. Toxic sediments tend to differentially affect the more sensitive species of the communities, even though the community structure may change seasonally. In other words, while the "players may change" the results of the game is generally the same.

33

Oxygen consumption  
and osmoregulation  
capacity

Although no interpretation of causality of treatment responses is attempted in our sublethal bioassays, the tests have been shown to be effective indicators of sublethal stress associated with sediment

Page

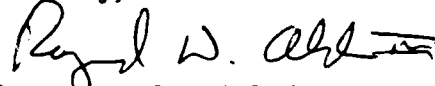
Topic

Comments

toxicity. Coupled with multivariate statistical models, these sublethal data are useful in classifying and "mapping" the sediments which produce biological effects but which are not out-and-out lethal to the bioassay organisms. Since these tests can be done during the standard SP and solid bioassays at very little additional costs, they provide a sensitive, cost effective approach in defining the relative toxicity of sediments. They have proven very useful in classifying moderately contaminated sediments which have the potential for affecting the overall health of biota exposed to them over the long term.

I hope that these comments will serve to clear up possible misunderstandings concerning our findings and philosophical position on bioassessment techniques. If you feel that there are any unresolved questions, please do not hesitate to contact me.

Sincerely,



Raymond W. Alden III, Ph.D.  
Director

RWA/reh

END

DTIC

9 - 86