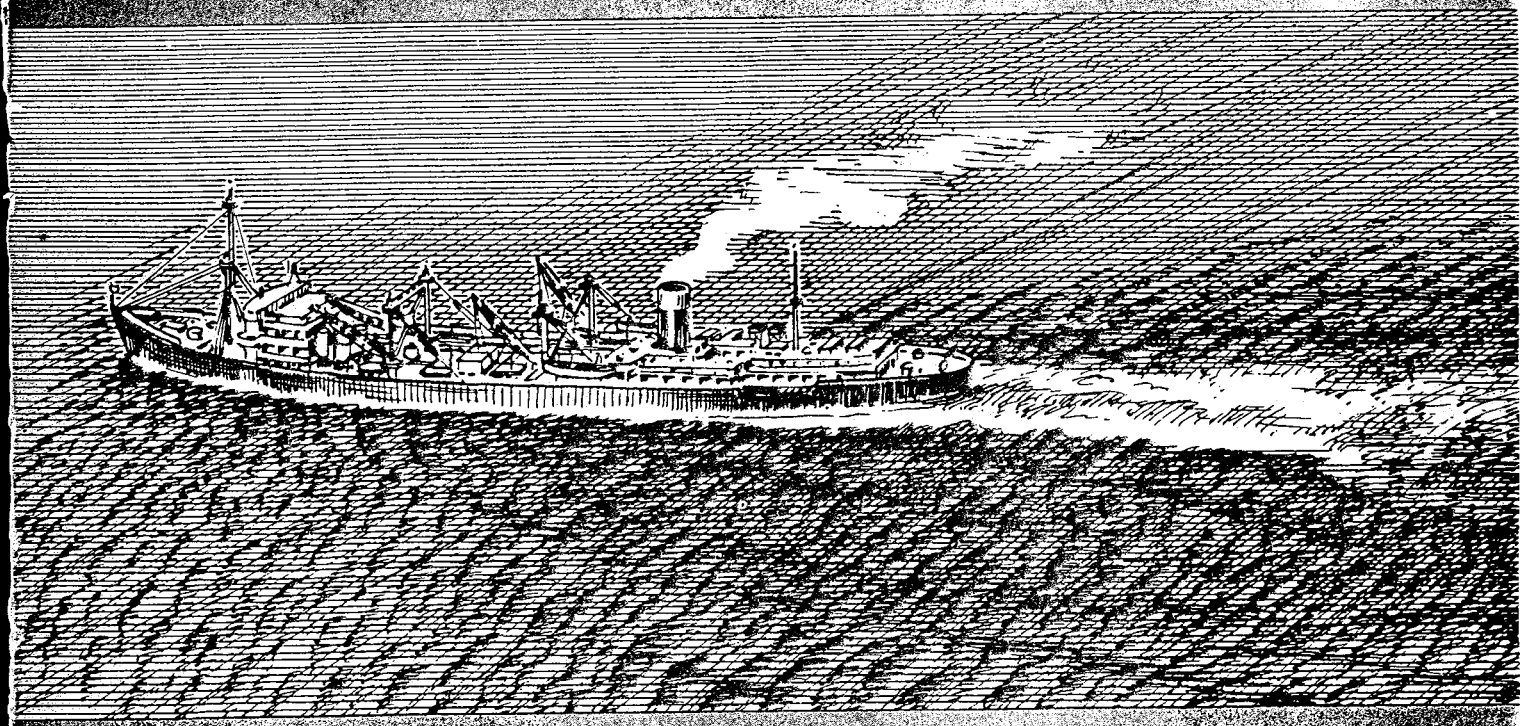
ENVIRONMENTAL PROTECTION AGENCY/ CORPS OF ENGINEERS TECHNICAL COMMITTEE ON CRITERIA FOR DREDGED AND FILL MATERIAL

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)

July 1977

(Second Printing April 1978)



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ENVIRONMENTAL PROTECTION AGENCY/ CORPS OF ENGINEERS TECHNICAL COMMITTEE ON CRITERIA FOR DREDGED AND FILL MATERIAL

TRANSMITTAL OF
"ECOLOGICAL EVALUATION OF PROPOSED DISCHARGE
OF DREDGED MATERIAL INTO OCEAN WATERS"
(Second Printing)

1. In accordance with Section 227.27(b) of the Federal Register, Vol. 42, No. 7, Tuesday, 11 January 1977, (referred to hereafter in this letter as the Register), an implementation manual has been developed jointly by the Environmental Protection Agency (EPA) and the Corps of Engineers (CE). This manual will be used in the implementation of Section 103 of Public Law 92-532, the Marine Protection, Research, and Sanctuaries Act of 1972. Procedures are presented for evaluation of potential environmental impacts of the discharge of dredged material into ocean waters, an evaluation that is required in considering permit applications for the transportation of dredged material for ocean dumping.

2. The manual transmitted herewith represents a multidisciplinary effort of both agencies to develop procedurally sound, routinely implementable guidance for complying with the technical requirements of the Register. The procedures given in the manual are applicable to evaluation of the potential ecological effects of dumping from hopper dredges, barges, and scows. The requirements of the Register are discussed, and detailed guidance is provided on sediment and water sample collection, preparation, and preservation; chemical analysis of the liquid phase; bioassays of liquid, suspended particulate, and solid phases; estimation of bioaccumulation potential; and the estimation of initial mixing.

3. The manual is not intended to establish standards or rigid criteria and should not be interpreted in such a manner. The document

attempts to provide a balance between the technical state-of-the-art and routinely implementable guidance for using the procedures specified in the Register and is intended to encourage continuity and cooperation between CE Districts and EPA Regions in evaluative programs for Section 103 permit activities. The manual is particularly important in forming a foundation to be augmented by more meaningful and comprehensive evaluation procedures and guidelines as these evolve from current and future environmental research. This second printing of the manual contains some minor modifications to the first edition that was published in July 1977. It is anticipated that the second edition of the manual will be published when new and more implementable evaluation procedures are developed and verified.

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PREFACE

According to Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1972), any proposed dumping of dredged material into ocean waters must be evaluated through the use of criteria published by the Environmental Protection Agency (EPA) in the Federal Register, Vol. 42, No. 7, Tuesday, 11 January 1977, and subsequently republished in Title 40 of the Code of Federal Regulations, Parts 220-228. These criteria state that an implementation manual describing the applicability of specific evaluative approaches and procedures will be developed jointly by EPA and the Corps of Engineers (CE). This manual contains those procedures considered applicable to evaluation of potential environmental impacts of the ocean disposal of dredged material, and it will be periodically revised and updated as advances in the technical state-of-the-art warrant.

By agreement of both agencies, this implementation manual was developed by the EPA/CE Technical Committee on Criteria for Dredged and Fill Material, co-chaired by Dr. Frank G. Wilkes of the EPA and Dr. Robert M. Engler of the CE. Due to the emphasis on bioassay in the Federal Register, much of the developmental input to the manual was from the Bioassay/Bioevaluation Subcommittee, co-chaired by Dr. Jack H. Gentile of the EPA and Dr. Richard K. Peddicord of the CE. Many individuals within both agencies contributed to the manual, with major input in various areas from those identified as follows:

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Appendix C - Liquid Phase Chemical Analyses:

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Appendix D - Guidance for Performing Liquid Phase and Suspended Particulate Phase Animal Bioassays:

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Appendix E - Guidance for Performing Liquid Phase and Suspended Particulate Phase Phytoplankton Bioassays:

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Appendix F - Guidance for Performing Solid Phase Bioassays:

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Appendix G - Guidance for Assessing Bioaccumulation Potential:

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Appendix H - Estimation of Initial Mixing:

Mr. Barry W. Holliday, EEL, WES, CE

Review of the manual was conducted by EPA through the Marine Protection Branch of the Oil and Special Materials Control Division, Office of Deputy Assistant Administrator for Health and Ecological Effects, and the Ocean Dumping Bioassay Committee and by the Corps of Engineers through the Office, Chief of Engineers, and the Environmental Effects Laboratory of the Waterways Experiment Station.

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PART I: INTRODUCTION

Background

1. Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972, Public Law (PL) 92-532, specifies that all proposed operations involving the transportation and dumping of dredged material into ocean waters must be evaluated to determine the potential environmental impact of such activities. This must be done by the Secretary of the Army and the Administrator of the Environmental Protection Agency (EPA) acting cooperatively through the District Engineer and Regional Administrator. Environmental evaluations must be in accordance with criteria published by EPA in the Federal Register, Vol. 42, No. 7, Tuesday, 11 January 1977, referred to as the Register throughout this implementation manual. As used in this manual, the term "Register" shall be synonymous with Title 40 of the Code of Federal Regulations, Parts 220-228, in which these criteria were republished following their initial publication in the Federal Register.

2. The primary intent of Section 103 of PL 92-532 is to regulate and limit adverse ecological effects of ocean dumping. Consequently, the Register emphasizes evaluative techniques such as bioassays and bioassessments, which provide relatively direct estimations of the potential for environmental impact. To conduct the required procedures properly requires considerable expertise in conducting biological evaluations. In addition, significant continuing effort and expense are required to collect and culture sufficient stocks of all the necessary species of organisms and maintain them in good condition in the laboratory to use whenever an evaluation must be conducted. There considerations argue against obtaining the services of a different group to conduct each evaluation. It is highly recommended that a few groups of demonstrated bioassay capability be selected, with each group conducting evaluations for a number of permit applications. This will enable these groups to develop adequate culturing and maintenance capabilities and the expertise and familiarity with the procedures required to consis-

tently conduct them properly and to provide the most reliable results at the least cost per evaluation.

Purpose and Scope

3. The Register specifies that this technical implementation manual for the criteria applicable to dredged material be developed jointly by EPA and the Corps of Engineers (CE). The manual was developed jointly by the EPA/CE Technical Committee on Criteria for Dredged and Fill Material, with the major contribution from the Bioassay/Bioevaluation Subcommittee. The manual is an attempt to provide a balance between technical state-of-the-art and routinely implementable guidance for using the evaluative procedures specified in the Register. Guidance is included on the appropriate uses and limitations of the various procedures and on sound interpretation of the results. Its structure follows the general order of test application and general priority of importance of testing and evaluation procedures presented in the Register.

4. This manual contains summaries and discussions of the procedures for ecological evaluation of dredged material required by the Register, tests to implement them, definitions, sample collection and preservation procedures, evaluative procedures, calculations, interpretative guidance, and supporting references required for the evaluation of permit applications in accordance with the Register. Even so, this manual cannot stand alone. It is imperative that the supporting references cited in each appendix be consulted for detailed or more comprehensive guidance whenever indicated. Before any evaluations are begun, the Register and this manual should be read in their entirety, and citations and references listed with the appendices should be consulted to obtain an understanding of the guidance the manual provides. The technical procedures in this manual were designed only for dredged material and should not be utilized for any other materials unless definitive research demonstrates their applicability.

5. This issue of the implementation manual contains evaluative procedures considered to be acceptable regulatory tools for most

situations. In some instances more sophisticated and complex biological evaluations may be warranted by special circumstances. However, variations of these procedures should be allowed only when the District Engineer and the Regional Administrator are able to justify and defend the technical validity of such variations. The field of ecological evaluation is a dynamic one, and new and better regulatory procedures are under development. As warranted by experience with this manual and the development of new procedures, the manual will be revised periodically. These revisions will be announced.

6. It should be emphasized that implementation of the criteria is the joint responsibility of the District Engineer and the Regional Administrator. This manual was developed by research personnel of both agencies to contain the best technical guidance available for implementation. However, it is inevitable that situations will arise that are not specifically addressed in the manual, as well as occasions when a choice of the appropriate course of action must be made. Such situations must be cooperatively worked out by the District Engineer and Regional Administrator to their mutual satisfaction as they occur.

7. This manual provides technical guidance to the fullest extent practical on implementation of the criteria. Yet technical evaluations can provide only part of the input to the decisionmaking process. Many of the criteria do not concern subjects amenable to quantitative evaluation. In such cases objective, qualitative decisions must be made. Indeed, the decision on granting of a permit is ultimately subjective. The criteria do not prohibit environmental change, but rather "unacceptable environmental impact." Consequently, for each permit application, the Regional Administrator and the District Engineer must decide how much potential impact is acceptable under the environmental, economic, social, and political conditions related to the operation in question. Technical and scientific evaluations provide an important but incomplete input to such decisions.

Applicability

8. This implementation manual is applicable to all activities involving the transportation of dredged material for the purpose of dumping it in ocean waters. These procedures do not apply to activities excluded in Section 220.1 of the Register. These criteria pertain to the transportation for ocean dumping of dredged material outside the baseline from which the territorial sea is measured.

Definitions

9. The following terms are briefly defined as used in this report and its appendices. See Section 220.2 and Part 227 Subpart G of the Register for complete definitions of terms used in the criteria.

Constituents. Chemical substances, solids, organic matter, and organisms associated with or contained in or on dredged material.

Criteria. Procedures and concepts published in the Register for the evaluation of dredged material ocean-dumping permit applications.

Disposal site. A precise geographical area within which ocean dumping of materials may be permitted. Includes both the bottom substrate and the water column within the specified boundaries.

Dredged material. Bottom sediment or material and the water associated with such sediment or material that have been dredged or excavated from the navigable waters of the United States.

Dumping. The disposition of material subject to the exclusions of paragraph 220.2(e) of the Register.

Initial mixing. Dispersion or diffusion of liquid, suspended particulate, and solid phases of dredged material that occurs within 4 hr after dumping.

Limiting permissible concentration (LPC) of:

a. Liquid phase: the concentration of dredged material that, after allowance for initial mixing, does not exceed applicable marine water-quality criteria or a toxicity threshold of 0.01 of the acutely toxic concentration.

b. Suspended particulate and solid phase: a concentration that will not cause unreasonable acute or chronic toxicity or sublethal adverse effects including bioaccumulation of toxic materials in the human food chain.

Liquid phase. The centrifuged and 0.45-μ-filtered supernatant remaining after 1 hr undisturbed settling of the mixture resulting from the vigorous 30-min agitation of a 1:4 ratio of dredged material and dredging site or disposal site water, as appropriate.

Ocean. Those waters of the open seas lying seaward of the baseline from which the territorial sea is measured (see paragraph 220.2(c) of the Register).

Solid phase. All material settling to the bottom within 1 hr in the liquid-phase procedure. (In practice, bottom sediments of in situ density may be considered to represent the solid phase.)

Suspended particulate phase. The supernatant, prior to centrifugation and filtration, obtained by the liquid-phase procedure.

Water-quality criteria. The criteria given for marine waters in the EPA publication "Quality Criteria for Water" as published in 1976 or in subsequent editions.

PART II: GENERAL APPROACHES FOR EVALUATION OF PERMIT APPLICATIONS

10. The potential effect of the ocean disposal of dredged material on marine organisms and human uses of the ocean may range from unmeasurable to important. These effects may differ at each disposal site and must be evaluated on a case-by-case basis. The Register provides criteria for such an evaluation, with an emphasis placed on direct assessment of biological impacts. The appropriate technical procedures are found in Parts 227 and 228 of the Register. These procedures and their relationship to each other are illustrated diagrammatically in Figure 1.

Applicability (Subpart A)

11. The Register recognizes that dredged material may behave differently from other materials that may be ocean dumped, but does not place all dredged material criteria in a separate section. Therefore, it is necessary to read Part 227, Subpart A, paragraph 227.1(b) carefully to determine those sections that are applicable to dredged material. It is these sections that are discussed in this manual.

Technical Evaluation (Subpart B)

12. The first evaluative consideration shown in Figure 1 involves the presence of certain substances that may not be ocean dumped under any circumstances. If any of these are present, the permit application must be denied without further consideration. Dredged materials, however, are highly unlikely to contain these substances and must usually receive the full technical evaluation required by the criteria.

13. There are obvious cases where dredged material is not considered chemically contaminated and would, therefore, cause negligible pollutional impact when discharged at an appropriate disposal site. Thus material that meets the requirements of paragraph 227.13(b) (see Appendix A, page A2) may be excluded from the technical evaluations

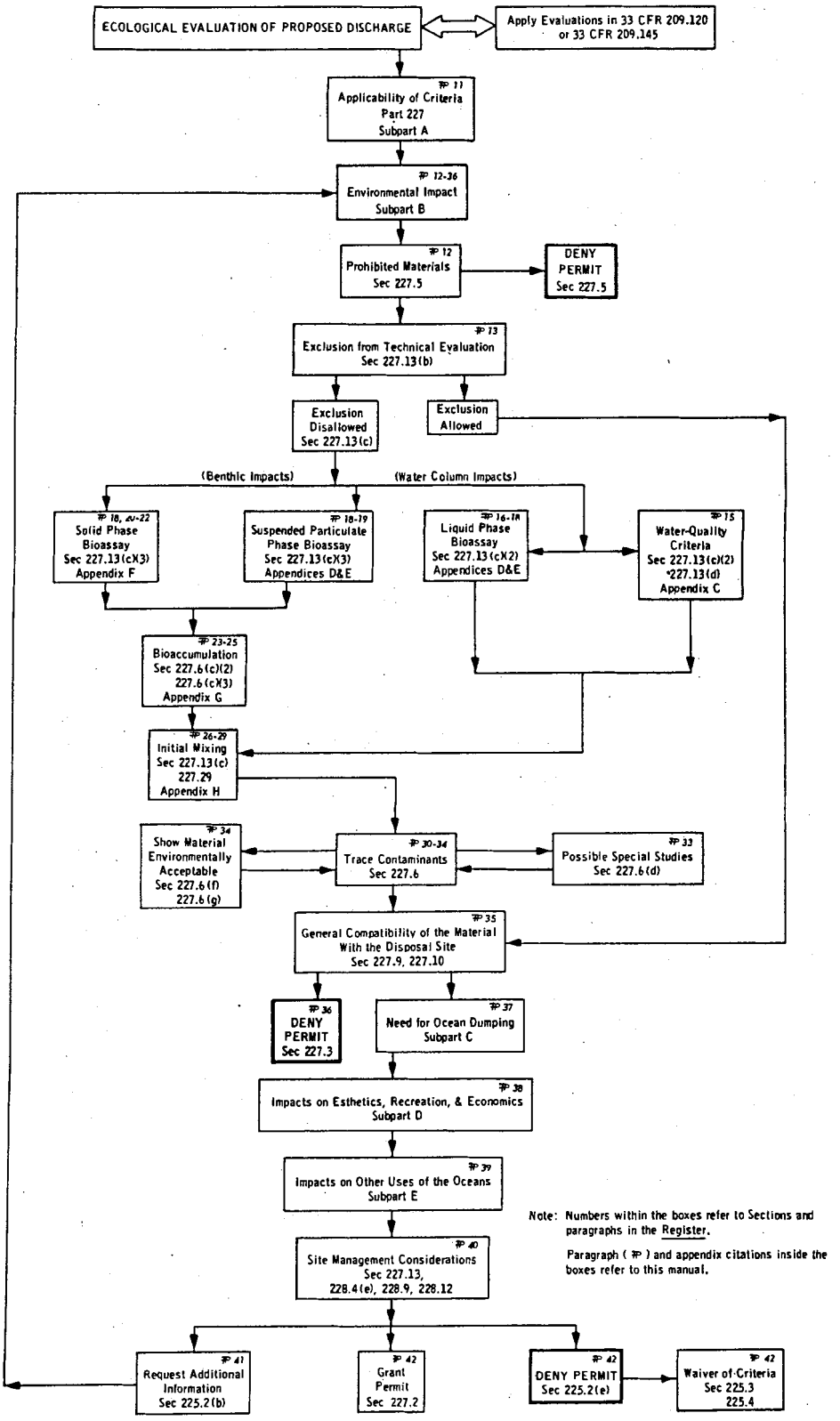

Figure 1. Sequence of testing and evaluation procedures

required by Section 227.13 and need be evaluated only in terms of its compatibility with the disposal site and the considerations of Subparts C, D, and E and the appropriate sections of Part 228, as illustrated in Figure 1.

14. Dredged material that does not meet the conditions for exclusion of paragraph 227.13(b) must receive a full technical evaluation of its potential for environmental impact. The evaluative procedures emphasize biological effects, rather than simple chemical presence, of possible contaminants. Dredged material is separated for evaluation into three phases, as defined in paragraph 227.32(b)(1) of the Register (see Appendix A, page A3). The liquid phase and the suspended particulate phase are considered to have the greatest potential for impact on the water column and are evaluated with this in mind. The solid phase has the greatest potential for impact on benthic organisms, and evaluative emphasis is placed there. All three phases must be evaluated, as indicated in Figure 1.

Liquid phase

15. The liquid phase of dredged material may be evaluated in either of two ways, as specified in paragraph 227.13(c)(2) of the Register. Where there is concern about specific contaminants that may be released in soluble form, the liquid phase may be analyzed chemically and the results evaluated by comparison to water-quality criteria for those contaminants after allowance for initial mixing. The period of initial mixing, discussed in paragraphs 26 and 27, must be allowed before comparing the predicted concentrations to water-quality criteria. This provides an indirect evaluation of potential biological impacts of the liquid phase, since the water-quality criteria were derived from bioassays of solutions of the various contaminants. Chemical evaluation of the liquid phase is possible only for those contaminants for which specific water-quality criteria have been established. The major constituents to be analyzed in the liquid phase are to be selected cooperatively by the District Engineer and the Regional Administrator, as discussed in paragraph 227.13(d) of the Register. Sample collection and preparation methods are given in Appendix B, and the appropriate

laboratory procedures and supporting references may be found in Appendix C of this manual.

16. If the water-quality criteria approach is not taken, the liquid phase must be evaluated by bioassays, as indicated in Figure 1. The direct bioassay approach is to be used when the liquid phase may contain major constituents not included in the water-quality criteria or when there is reason to be concerned about possible synergistic effects of certain contaminants. In these cases liquid phase bioassays can aid in evaluating the importance and the total net impact of dissolved chemical constituents released from the sediment during disposal operations.

17. Liquid phase bioassays must be conducted with "appropriate sensitive marine organisms." Paragraph 227.27(c) of the Register (see Appendix A, page A8) defines this to mean at least three species consisting of one phytoplankton or zooplankton species, one crustacean or mollusc, and one fish. Phytoplankton bioassays can indicate the potential for algal stimulation or inhibition by the dredged material in question. However, it is widely felt that potential effects on phytoplankton are generally of little environmental concern at ocean dredged material disposal sites, due to the extremely dynamic and variable characteristics of natural phytoplankton assemblages and to the rapid mixing and dilution that occurs in the water column. For these reasons, unless there is a specific reason to be concerned about potential effects of the proposed operation on phytoplankton, it is recommended that a zooplankton species be selected to fulfill that portion of the bioassay species requirement. Laboratory procedures for conducting liquid-phase animal bioassays may be found in Appendix D; guidance on phytoplankton bioassays, if they are felt necessary, is contained in Appendix E of this manual.

18. It should be recognized that dredged material bioassays cannot be considered precise predictors of environmental effects. They must be regarded as quantitative estimators of those effects, making interpretation somewhat subjective. In order to avoid adding more uncertainty to their interpretation, the animal bioassays given in this manual all utilize mortality as an end point. The significance of this response

to the individuals involved is clear, but the state of ecological understanding is such that it remains impossible to predict the ecological consequences of the death of a given percent of the local population of a particular species. For example, there is presently no basis for estimating whether the loss at the disposal site of 10 percent of a particular crustacean species would have inconsequential or major ecological effects. This interpretative uncertainty becomes overpowering when a parameter whose ecological meaning is not as clear as mortality is used as the bioassay end point. In view of the interpretative difficulties, the bioassays in this manual specify death as the response to be measured. Interpretative guidance does not attempt to consider the ecological meaning of the mortality observed, but takes the environmentally protective approach prescribed in the Register that any statistically significant increase in mortality compared to the controls is potentially undesirable. It is important to realize, however, that a statistically significant effect in a laboratory bioassay cannot be taken as a prediction that an ecologically important impact would occur in the field. Bioassay results must be evaluated in light of initial mixing (Figure 1) as discussed in paragraphs 26 through 28.

Suspended particulate phase

19. The suspended particulate phase of dredged material may be evaluated for potential environmental impact only by use of bioassays. No chemical procedure has yet been devised that will determine the amount of environmentally active contaminants present in the suspended particulate phase of dredged material. Therefore, bioassays are used to evaluate directly the potential for biological impacts due to both the physical presence of suspended particles and to any biologically active contaminants associated with the particulates and/or the dissolved fraction. Suspended particulate phase bioassays must also be conducted with appropriate sensitive marine organisms, as described in paragraph 17 for liquid phase bioassays. In addition to the discussion there concerning the general advisability of phytoplankton bioassays with dredged material, it should be noted that suspended particulate phase phytoplankton bioassays are extremely difficult to conduct and

interpret validly. This is due to interferences and predation on the test species by protozoans contained in the dredged material being tested. Consequently, in most cases the maximum amount of useful information on potential effects of the proposed disposal operation will be obtained by bioassaying zooplankton, a crustacean or mollusc, and a fish.

Solid phase

20. It is generally felt that if a dredged material is going to have an environmental impact, the greatest potential for impact lies in the solid phase. This is because it is not mixed and dispersed as rapidly or as greatly as the liquid and suspended particulate phases, and bottom-dwelling animals live and feed in and on the deposited solid phase for extended periods. Therefore, unless there is reason to do otherwise, the major evaluative efforts should be placed on the solid phase. The Register requires that bioassays be used to evaluate the potential impact of the solid phase. No chemical procedures exist that will determine the environmental activity of any contaminants or combination of contaminants present in the solid phase of dredged material. Therefore, animals are used in a bioassay to provide a measurement of environmental activity of the chemicals found in the material.

21. Solid phase bioassays, described in Appendix F, must be conducted with "appropriate sensitive benthic marine organisms." Paragraph 227.27(d) of the Register (Appendix A, page A8) defines this to mean at least three species, consisting of one filter-feeding, one deposit-feeding, and one burrowing species. These are broad overlapping general categories, and it is recommended that the species be selected to include a crustacean, an infaunal bivalve, and an infaunal polychaete.

22. Paragraph 18 is a key discussion pertinent to all bioassay procedures in this manual, including solid phase bioassays, which also measure mortality as the end point because of its clear physiological significance. However, as pointed out in paragraph 18, the ecological meaning of the death of a given percent of the animals of one or several species at the disposal site cannot be estimated at present. Therefore, the interpretative guidance presented for the solid phase bioassay is

environmentally protective in that any statistically significant increase in mortality compared to the controls is considered potentially undesirable. This approach is environmentally conservative in that it does not attempt to consider the ecological meaning of the mortality observed, but assumes that any mortality might be adverse. Again, it must be emphasized that a statistically significant effect in a laboratory bioassay does not necessarily imply that an ecological important impact would occur in the field. Solid phase bioassay results must also be interpreted in light of initial mixing as described in paragraph 28.

Bioaccumulation

23. The criteria require that all biological evaluations of the suspended particulate and solid phases include an assessment of the potential for contaminants from the dredged material to be bioaccumulated in the tissues of marine organisms (Figure 1 and paragraphs 227.6 (c)(2) and (3) of the Register). This is intended to assess the potential for the long-term accumulation of toxins in the food web to levels that might be harmful to the ultimate consumer, which is often man, without killing the intermediate organisms. In order to use bioaccumulation data in a permitting decision, it is necessary to predict whether there will be a cause-and-effect relationship between the animals' presence in the dredged material and a meaningful elevation of body burdens of contaminants above those of similar animals not in the dredged material.

24. Since concern about bioaccumulation is focused on the possibility of gradual uptake over long exposure times, primary attention is usually given to the solid phase that is deposited on the bottom. Bioaccumulation from the suspended particulate phase is considered to be of secondary concern except in special cases, due to the short exposure time resulting from rapid dispersion of the suspended particulates by mixing. Should this be a major consideration for the operation in question, the laboratory bioaccumulation procedures given in Appendix G may be used to assess the suspended particulate phase. Because of the long-term nature of the concerns, bioaccumulation from the solid phase is at present best evaluated in the field where possible. This can be done

only when an historical precedent exists for the proposed operation, as discussed in paragraphs 2 and 3 of Appendix G. Under these conditions, a field assessment provides the most useful information because the animals have been exposed to the sediment under natural conditions for longer periods than are now generally practical in the laboratory. To the extent that source control has prevented increased input of contaminants, it will generally be true that sediment quality at dredging sites will not be lower than at the time of previous dredging and disposal operations. Therefore, since the same disposal site is traditionally used repeatedly for each dredging site, a valid historical precedent probably exists at present for most disposal operations utilizing sites designated in Section 228.12 of the Register.

25. The environmental interpretation of bioaccumulation data is even more difficult than for bioassays because in most cases it is impossible to quantify either the ecological consequences of a given tissue concentration of a constituent that is bioaccumulated or even the consequences of that body burden to the animal whose tissues contain it. Almost without exception in the marine environment, there is no technical basis for establishing, for example, the tissue concentration of copper in a species of polychaete that would be detrimental to that organism, not to mention the impossibility of estimating the effect of that organism's body burden on a predator. Therefore, in order to ensure environmental safety, the interpretative guidance assumes that any statistically significant bioaccumulation relative to animals not in dredged material, but living in material of similar sedimentological character, is potentially undesirable. The evaluation of experimental results using this approach requires the user to recognize the fact that a statistically significant difference cannot be presumed to predict the occurrence of an ecologically important impact. Bioaccumulation results must also be evaluated in light of initial mixing as discussed in the next section.

Initial mixing

26. All data from chemical analysis of the liquid phase, bioassays, and bioaccumulation studies must be interpreted in light of initial

mixing, as illustrated in Figure 1. This is necessary since biological effects (which are the basis for water-quality criteria) are a function of biologically available contaminant concentration and exposure time of the organism. Laboratory bioassays expose organisms to relatively constant concentrations for fixed periods of time, while in the field both concentration and exposure time to a particular concentration change continuously. Since both factors will influence the degree of biological impact, it is necessary to incorporate the mixing expected at the site in the interpretation of biological data.

27. Initial mixing is defined in Section 227.29 of the Register (Appendix A, page A6) and guidance on estimation of initial mixing may be found in Appendix H of this manual. Methods for incorporation of mixing estimations into the interpretation of water-quality results are included in Appendix C, and these methods for liquid and suspended particulate phase bioassay data are included in Appendix D.

28. Although the Register requires the consideration of initial mixing and dispersion of the sediment after it reaches the bottom in interpreting solid phase bioassay data, no objective method of doing so has been devised. Rather, there has been an attempt to incorporate the phenomenon of solid phase sediment dispersion into the bioassay design to some extent. The concept is expressed in the environmental impact statement on the ocean dumping criteria* that "EPA has chosen to allow some change in sediment characteristics or water chemistry as being reasonable, but no damage to the biota out-side the region of initial mixing is allowed under these criteria." The solid phase bioassay technique, therefore, does not evaluate the physical effects of massive sediment deposition immediately under the discharging vessel, since the primary concern is that damage not extend beyond the region of initial mixing. Instead, the technique generally approximates conditions near the disposal site boundary where sediment dispersion has reduced the

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* U. S. Environmental Protection Agency, "Proposed Revision to Ocean Dumping Criteria: Final Environmental Impact Statement," 1977, Office of Water Program Operations, Washington, DC, p 128.

depth of deposited dredged material to a few centimetres. The solid phase bioassay technique measures the effects of chemicals associated with this deposited sediment, rather than physical effects of the sediment. It is apparent that there will be a gradient of decreasing effects with increasing distance away from the disposal site due to dispersion, although the nature of this gradient cannot be determined. Therefore, the environmentally protective assumption is made that a statistically significant effect in the solid phase bioassay indicates a real potential for environmental impact from the solid phase.

29. The Register also requires consideration of initial mixing in interpreting the results of bioaccumulation studies. In contrast to the situation with liquid and suspended particulate phase bioassays, no objective semiquantitative method for incroporating mixing and dilution into the interpretation of results has been developed for bioaccumulation data. If, in light of paragraph 24, bioaccumulation from the suspended particulate phase is of concern, evaluation of the results must subjectively consider the effects of mixing on exposure time and concentration, and thus on bioaccumulation. In field evaluations of bioaccumulation potential, mixing is fully incorporated into the experimental design because the animals have lived in the sediment under the natural conditions at the site since the previous disposal operation. This is a major advantage of field assessment of bioaccumulation potential from the solid phase over laboratory evaluations.

Trace contaminants

30. As illustrated in Figure 1, the presence or absence of trace contaminants must be determined for all three phases of the material. Section 227.6 is perhaps the key section of the criteria, since dredged material may not be ocean dumped if it contains any of the listed substances in greater than trace amounts. This is not defined in terms of numerical chemical limits whose environmental meaning is uncertain, but rather* "...EPA came to the conclusion that the basis for regulation {of trace contaminants} should be the probable impact of these constituents

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* ibid., p 83.

on the biota and that the measurement technique used should be bioassays on the waste itself." Section 227.6(b) of the Register expresses in regulatory language the idea that trace concentrations be defined as those too low to cause an environmental effect.

31. In other words, marine organisms are regarded, in a sense, as analytical instruments for determining the environmentally active portions of any contaminants present. Implementation of this approach to the definition of trace contaminants requires that lack of effect in bioassays and bioaccumulation studies be taken to mean that contaminants are absent or present only in amounts and/or forms that are not environmentally active, and therefore do not exceed so-called "trace concentrations." When effects do occur in dredged material bioassays, it is not possible within the present state-of-knowledge to determine which constituent(s) caused the observed effects. Therefore, it must be assumed they are due to Section 227.6 materials because it cannot be established that this is not the case. This would mean some contaminant(s) is present in greater than trace concentrations. In practice, the exact identity of the contaminant(s) causing the effect is of little concern from a regulatory viewpoint, since dredged material that might cause an environmental effect for any reason should not be ocean dumped except perhaps under special circumstances. Following this reasoning, bioaccumulation of any potentially harmful constituent, whether listed in Section 227.6 or not, could make the material undesirable for ocean dumping.

32. Since the assessment of trace contaminants depends upon the determination of effects, it cannot be made until the bioassays (and/or water-quality studies), and bioaccumulation studies are completed and interpreted with consideration of mixing. Only then can effects, and thus the presence of contaminants in other than trace concentrations, be estimated. This sequence is illustrated in Figure 1.

33. Paragraph 227.6(d) allows special studies to estimate the potential environmental impact of materials believed to contain compounds identified as carcinogens, mutagens, or teratogens for which there are no water-quality criteria. This paragraph is expected to

apply to relatively few permit applications for the ocean disposal of dredged material. In cases where it does apply, the required special studies would have to be specifically designed for the contaminant of concern under the particular conditions of the operation in question. Such highly site- and problem-specific studies are beyond the scope of a manual such as this and must be designed for each situation in which they are needed.

34. The prohibitions and limitations of Section 227.6 do not apply when it can be demonstrated that the material in question is environmentally acceptable, as described in paragraphs 227.6(f) or (g) of the Register (Appendix A). Again, the studies necessary to demonstrate compliance with these paragraphs would have to be designed specifically for the environmental conditions and contaminants in question, making them so site specific as to be beyond the scope of this manual. Both these studies and those discussed in paragraph 33 would have to be designed cooperatively by the District Engineer and the Regional Administrator to satisfy their mutual concerns about compliance of the material with the criteria.

General compatibility with the disposal site

35. Once the preceding criteria have been satisfied, the general compatibility of the dredged material with the proposed disposal site must be evaluated under Sections 227.9 and 227.10 of the Register. Both sections are rather subjective criteria, and no specific evaluative procedures exist for determining compliance with either section. It should be noted, however, that the available evidence indicates that the amounts of dredged material usually ocean dumped at one time and place generally would not create long-term damage caused simply by the volume of material dumped. Notice from Figure 1 that dredged material excluded from technical evaluation under paragraph 227.13(b) must meet the requirements of Sections 227.9 and 227.10.

Evaluation of Subpart B results

36. At this point the evaluations required under Subpart B of the Register will have been conducted. Under Section 227.3, if a dredged material fails to meet the Subpart B criteria, the permit may be denied.

Need for Ocean Dumping (Subpart C)

37. No material may be ocean dumped unless there is a demonstrated need to do so under Subpart C. This subpart is in effect an evaluation of alternative disposal sites in terms of potential environmental impacts, irreversible commitment of resources, and costs. No disposal alternative is initially considered more desirable than any other and the evaluation is to be made on a case-by-case basis. That is, confined or upland disposal cannot be considered environmentally preferable to aquatic disposal unless consideration of the potential environmental impacts (including groundwater contamination, leachate and runoff impacts, and permanent alteration of the site) show it to be so. Similarly, ocean disposal should not automatically be considered the most desirable alternative. As pointed out in Section 227.14, specific quantitative criteria for evaluating the need for ocean dumping cannot be given, and this evaluation remains essentially a subjective one.

Impacts on Esthetics, Recreation, and Economics (Subpart D)

38. Before a permit may be granted, the probable impacts on esthetics, recreational, and economic values must be evaluated, as described in Subpart D. Although this, too, is a nonquantitative evaluation, consideration of information from the Subpart B technical assessments is required in paragraph 227.18. Despite the qualitative nature of the required assessment, paragraph 227.19 requires that the results be expressed, insofar as possible, in quantitative terms.

Impacts on Other Uses of the Ocean (Subpart E)

39. Subpart E is related to the above requirements, but it requires evaluation of specific actual or potential uses of the disposal site, including but not limited to those listed in paragraph 227.21. These again are criteria for which specific quantitative tests of compliance cannot be given. However, much information developed in the

Subpart B technical evaluations will be directly relevant to the assessment of potential impacts on living resources and their utilization.

Site Management Considerations

40. The evaluation of the proposed disposal site in relation to requirements for effective site management must also be considered, according to paragraph 227.13(a). This is covered in Part 228 of the Register, of which only paragraph 228.4(e) and Sections 228.9 and 228.12 apply to dredged material. Specific implementation procedures for these requirements cannot be offered at present, and appropriate techniques to satisfy the criteria will have to be worked out cooperatively by the Regional Administrator and the District Engineer on a case-by-case basis.

Decision on the Permit Application

41. It is possible that in some cases adequate information upon which to base a sound environmental evaluation of a permit under the criteria will not be supplied. In such cases paragraph 225.2(b) allows additional information to be requested and the application to be reevaluated.

42. Only when dredged material can comply with all the applicable requirements of Parts 227 and 228, as discussed earlier, may a permit be granted for ocean dumping under paragraph 227.2. The permit must be denied in all other cases. If the permit is denied but the dredging is essential and no feasible alternatives are available, a waiver of the criteria may be sought under Sections 225.3 and 225.4.

Appendix A: Reorganization of Section 227.13 from "Ocean Dumping—Final Revisions of Regulations and Criteria"* to Incorporate Cross-References

This appendix is appropriate for use by all regulatory elements concerned with the ocean disposal of dredged material. It is a compilation and reorganization of those sections of Part 227 of the 11 January 1977 Federal Register that concern technical evaluation of dredged material proposed for ocean disposal. There have been no alterations of content, but the sections have simply been rearranged to incorporate cross-referenced items. This appendix concerns technical evaluation only and must be used in conjunction with the other pertinent sections of the Federal Register.

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* Environmental Protection Agency, "Ocean Dumping—Final Revisions of Regulations and Criteria," Federal Register, Part VI, Vol. 42, No. 7, Tuesday, 11 January 1977.

§ 227.1 Applicability.

(a) Section 102 of the Act requires that criteria for the issuance of ocean disposal permits be promulgated after consideration of the environmental effect of the proposed dumping operation, the need for ocean dumping, alternatives to ocean dumping, and the effect of the proposed action on esthetic, recreational and economic values, and on other uses of the ocean. This Part 227 and Part 228 of this Subchapter H together constitute the criteria established pursuant to Section 102 of the Act. The decision of the Administrator, Regional Administrator or the District Engineer, as the case may be, to issue or deny a permit and to impose specific conditions on any permit issued will be based on an evaluation of the permit application pursuant to the criteria set forth in this Part 227 and upon the requirements for disposal site management pursuant to the criteria set forth in Part 228 of this Subchapter H.

(b) With respect to the criteria to be used in evaluating disposal of dredged materials, this Section 227.1 and Subparts C, D, E, and G apply in their entirety. To determine whether the proposed dumping of dredged material complies with Subpart B, only Sections 227.4, 227.5, 227.6, 227.9, 227.10, and 227.13 apply. An applicant for a permit to dump dredged material must comply with all of Subparts C, D, E, G, and applicable sections of B, to be deemed to have met the EPA criteria for dredged material dumping promulgated pursuant to Section 102(a) of the Act. If, in any case, the Chief of Engineers finds that, in the disposition of dredged material, there is no economically feasible method or site available other than a dumping site, the utilization of which would result in noncompliance with the criteria established pursuant to Subpart B relating to the effects of dumping or with the restrictions established pursuant to Section 102(c) of the Act relating to critical areas, he shall so certify and request that the Secretary of the Army seek a waiver from the Administrator pursuant to Part 225.

(c) The Criteria of this Part 227 are established pursuant to Section 102 of the Act and apply to the evaluation of proposed dumping of materials under Title I of the Act. The Criteria of this Part 227 deal with the evaluation of proposed dumping of materials on a case-by-case basis from information supplied by the applicant or otherwise available to EPA or the Corps of Engineers concerning the characteristics of the waste and other considerations relating to the proposed dumping.

(d) After consideration of the provisions of Sections 227.28 and 227.29, no permit will be issued when the dumping would result in a violation of applicable water quality standards.

§ 227.13 Dredged materials.

(a) Dredged materials are bottom sediments or materials that have been dredged or excavated from the navigable waters of the United States, and their disposal into ocean waters is regulated by the U. S. Army Corps of Engineers using the criteria of applicable sections of Parts 227 and 228. Dredged material consists primarily of natural sediments or materials which may be contaminated

by municipal or industrial wastes or by runoff from terrestrial sources such as agricultural lands.

(b) Dredged material which meets the criteria set forth in the following paragraphs (1), (2), or (3) is environmentally acceptable for ocean dumping without further testing under this section:

(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) When:

(i) The material proposed for dumping is substantially the same as the substrate at the proposed disposal site; and

(ii) The site from which the material proposed for dumping is to be taken is far removed from known existing and historical sources of pollution so as to provide reasonable assurance that such material has not been contaminated by such pollution.

(c) When dredged material proposed for ocean dumping does not meet the criteria of paragraph (b) of this section, further testing of the liquid, suspended particulate, and solid phases, as defined in Section 227.32, is required . . . .

§ *227.32 Liquid, suspended particulate, and solid phases of a material.*

(a) For the purposes of these regulations, the liquid phase of a material, subject to the exclusions of paragraph (b) of this section, is the supernatant remaining after one hour undisturbed settling, after centrifugation and filtration through a 0.45 micron filter. The suspended particulate phase is the supernatant as obtained above prior to centrifugation and filtration. The solid phase includes all material settling to the bottom in one hour. Settling shall be conducted according to procedures approved by EPA.

(b) For dredged material, other material containing large proportions of insoluble matter, materials which may interact with ocean water to form insoluble matter or new toxic compounds, or materials which may release toxic compounds upon deposition, the Administrator, Regional Administrator, or the District Engineer, as the case may be, may require that the separation of liquid, suspended particulate, and solid phases of the material be performed upon a mixture of the waste with ocean water rather than on the material itself. In such cases the following procedures shall be used:

(1) For dredged material, the liquid phase is considered to be the centrifuged and 0.45 micron filtered supernatant remaining after one hour undisturbed settling of the mixture resulting from a vigorous 30-minute agitation of one part bottom sediment from the dredging site with four parts water (vol/vol) collected from the dredging site or from the disposal site, as appropriate for the type of dredging operation. The suspended particulate phase is the supernatant as obtained above prior to centrifugation and filtration. The solid phase is considered to be all material settling to the bottom within one hour. Settling shall be conducted by procedures approved by EPA and the Corps of Engineers.

(2) For other materials, the proportion of ocean water used shall be the minimum amount necessary to produce the anticipated effect (e.g., complete neutralization of an acid or alkaline waste) based on guidance provided by EPA on particular cases, or in accordance with approved EPA procedures. For such material the liquid phase is the filtered and centrifuged supernatant resulting from the mixture after 30 minutes of vigorous shaking followed by undisturbed settling for one hour. The suspended particulate phase is the supernatant as obtained above prior to centrifugation and filtration. The solid phase is the insoluble material settling to the bottom in that period.

§ 227.13(c) Continued

. . .Based on the results of such testing, dredged material can be considered to be environmentally acceptable for ocean dumping only under the following conditions:

(1) The material is in compliance with the requirements of Section 227.6; and. . .

§ *227.6 Constituents prohibited as other than trace contaminants.*

(a) Subject to the exclusion of paragraphs (f), (g), and (h) of this section, the ocean dumping, or transportation for dumping, of materials containing the following constituents as other than trace contaminants will not be approved on other than an emergency basis:

(1) Organohalogen compounds;

(2) Mercury and mercury compounds;

(3) Cadmium and cadmium compounds;

(4) Oil of any kind or in any form, including but not limited to petroleum, oil sludge, oil refuse, crude oil, fuel oil, heavy diesel oil, lubricating oils, hydraulic fluids, and any mixtures containing these, transported for the purpose of dumping insofar as these are not regulated under the FWPCA;

(5) Known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion.

(b) These constituents will be considered to be present as trace contaminants only when they are present in materials otherwise acceptable for ocean dumping in such forms and amounts in liquid, suspended particulate, and solid phases that the dumping of the materials will not cause significant undesirable effects, including the possibility of danger associated with their bioaccumulation in marine organisms.

(c) The potential for significant undesirable effects due to the presence of these constituents shall be determined by application of results of bioassays on liquid, suspended particulate, and solid phases of wastes according to procedures acceptable to EPA, and for dredged material, acceptable to EPA and the Corps of Engineers. Material shall be deemed environmentally acceptable for ocean dumping only when the following conditions are met:

(1) The liquid phase does not contain any of these constituents in concentrations which will exceed applicable marine water quality criteria after allowance for initial mixing; provided that mercury concentrations in the disposal site, after allowing for initial mixing, may exceed the average normal ambient concentrations of mercury in ocean waters at or near the dumping sites which would be present in the absence of dumping, by not more than 50 percent; and

(2) Bioassay results on the suspended particulate phase of the waste do not indicate occurrance of significant mortality or significant adverse sublethal effects including bioaccumulation due to the dumping of wastes containing the constituents listed in paragraph (a) of this section. These bioassays shall be conducted with appropriate sensitive marine organisms as defined in Section 227.27(c) using procedures for suspended particulate phase bioassays approved by EPA, or, for dredged material, approved by EPA and the Corps of Engineers. Procedures approved for bioassays under this section will require exposure of organisms for a sufficient period of time and under appropriate conditions to provide reasonable assurance, based on consideration of the statistical significance of effects at the 95 percent confidence level, that, when the materials are dumped, no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation of the constituents listed in paragraph (a) of this section; and

(3) Bioassay results on the solid phase of the wastes do not indicate occurrence of significant mortality or significant adverse sublethal effects due to the dumping of wastes containing the constituents listed in paragraph (a) of this section. These bioassays shall be conducted with sensitive benthic organisms using benthic bioassay procedures approved by EPA, or, for dredged material, approved by EPA and the Corps of Engineers. Procedures approved for bioassays under this section will require exposure of organisms for a sufficient period of time to provide reasonable assurance, based on considerations of statistical significance of effects at the 95 percent confidence level, that, when the materials are dumped, no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation of the constituents listed in paragraph (a) of this section; and

(4) For persistent organohalogens not included in the applicable

marine water quality criteria, bioassay results on the liquid phase of the waste show that such compounds are not present in concentrations large enough to cause significant undesirable effects due either to chronic toxicity or to bioaccumulation in marine organisms after allowance for initial mixing.

(d) When the Administrator, Regional Administrator or District Engineer, as the case may be, has reasonable cause to believe that a material proposed for ocean dumping contains compounds identified as carcinogens, mutagens, or teratogens for which criteria have not been included in the applicable marine water quality criteria, he may require special studies to be done prior to issuance of a permit to determine the impact of disposal on human health and/or marine ecosystems. Such studies must provide information comparable to that required under paragraph (c) (3) of this section.

(e) The criteria stated in paragraphs (c) (2) and (3) of this section will become mandatory as soon as announcement of the availability of acceptable procedures is made in the FEDERAL REGISTER. At that time the interim criteria contained in this Section 227.6(e) shall no longer be applicable.

NOTE: The remainder of this paragraph has been made inapplicable by the notice of "Availability of Implementation Manual, 'Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters,'" Federal Register, Vol. 42, No. 7, 7 September 1977, page 44835.

(f) The prohibitions and limitations of this section do not apply to the constituents identified in paragraph (a) of this section when the applicant can demonstrate that such constituents are (1) present in the material only as chemical compounds or forms (e.g., inert insoluble solid materials) non-toxic to marine life and non-bioaccumulative in the marine environment upon disposal and thereafter, or (2) present in the material only as chemical compounds or forms which, at the time of dumping and thereafter, will be rapidly rendered non-toxic to marine life and non-bioaccumulative in the marine environment by chemical or biological degradation in the sea; provided they will not make edible marine organisms unpalatable; or will not endanger human health or that of domestic animals, fish, shellfish, or wildlife.

(g) The prohibitions and limitations of this section do not apply to the constituents identified in paragraph (a) of this section for the granting of research permits if the substances are rapidly rendered harmless by physical, chemical or biological processes in the sea; provided they will not make edible marine organisms unpalatable and will not endanger human health or that of domestic animals.

§ 227.13(c) Continued

. . .(2)(i) All major constituents of the liquid phase are in compliance with the applicable marine water quality criteria after allowance for initial mixing; or. . .

§ *227.31 Applicable marine water quality criteria.*

Applicable marine water quality criteria means the criteria given for marine waters in the EPA publication "Quality Criteria for Water" as published in 1976 and amended by subsequent supplements or additions.

§ *227.29 Initial mixing.*

(a) Initial mixing is defined to be that dispersion or diffusion of liquid, suspended particulate, and solid phases of a waste which occurs within four hours after dumping. The limiting permissible concentration shall not be exceeded beyond the boundaries of the disposal site during initial mixing, and shall not be exceeded at any point in the marine environment after initial mixing. The maximum concentration of the liquid, suspended particulate, and solid phases of a dumped material after initial mixing shall be estimated by one of these methods, in order of preference:

(1) When field data on the proposed dumping are adequate to predict initial dispersion and diffusion of the waste, these shall be used, if necessary, in conjunction with an appropriate mathematical model acceptable to EPA or the District Engineer, as appropriate.

(2) When field data on the dispersion and diffusion of a waste of characteristics similar to that proposed for discharge are available, these shall be used in conjunction with an appropriate mathematical model acceptable to EPA or the District Engineer, as appropriate.

(3) When no field data are available, theoretical oceanic turbulent diffusion relationships may be applied to known characteristics of the waste and the disposal site.

(b) When no other means of estimation are feasible,

(1) The liquid and suspended particulate phases of the dumped waste may be assumed to be evenly distributed after four hours over a column of water bounded on the surface by the release zone and extending to the ocean floor, thermocline, or halocline if one exists, or to a depth of 20 meters, whichever is shallower, and

(2) The solid phase of a dumped waste may be assumed to settle rapidly to the ocean bottom and to be distributed evenly over the ocean bottom in an area equal to that of the release zone as defined in Section 227.28. . . .

§ *227.28 Release zone.*

The release zone is the area swept out by the locus of points constantly 100 meters from the perimeter of the conveyance engaged in dumping activities, beginning at the first moment in which dumping is scheduled to occur and ending at the last moment in which dumping is scheduled to occur. No release zone shall exceed the total surface area of the dumpsite.

§ *227.29 Continued*

. . .(c) When there is reasonable scientific evidence to demonstrate that other methods of estimating a reasonable allowance for initial mixing are appropriate for a specific material, such methods may be used with the concurrence of EPA after appropriate scientific review.

§ 227.13(c) **Continued**

. . .(ii) When the liquid phase contains major constituents not included in the applicable marine water criteria, or there is reason to suspect synergistic effects of certain contaminants, bioassays on the liquid phase of the dredged material show that it can be discharged so as not to exceed the limiting permissible concentration as defined in paragraph (a) of Section 227.27; and. . .

§ *227.27 Limiting permissible concentration (LPC).*

(a) The limiting permissible concentration of the liquid phase of a material is:

(1) That concentration of a constituent which, after allowance for initial mixing as provided in Section 227.29, does not exceed applicable marine water quality criteria; or, when there are no applicable marine water quality criteria,

(2) That concentration of waste or dredged material in the receiving water which, after allowance for initial mixing, as specified in Section 227.29, will not exceed a toxicity threshold defined as 0.01 of a concentration shown to be acutely toxic to appropriate sensitive marine organisms in a bioassay carried out in accordance with approved EPA procedures.

(3) When there is reasonable scientific evidence on a specific waste material to justify the use of an application factor other than 0.01 as specified in paragraph (a)(2) of this section, such alternative application factor shall be used in calculating the LPC.

(b) The limiting permissible concentration of the suspended particulate and solid phases of a material means that concentration which will not cause unreasonable acute or chronic toxicity or other sublethal adverse effects based on bioassay results using appropriate sensitive marine organisms in the case of the suspended particulate phase, or appropriate sensitive benthic marine organisms in the case of the solid phase; or which will not cause accumulation of toxic materials in the human food chain. These bioassays are to be conducted in accordance with procedures approved by EPA, or, in the case of dredged material, approved by EPA and the Corps of Engineers (for instance, the procedure contained in this manual).

(c) "Appropriate sensitive marine organisms" means at least one species each representative of phytoplankton or zooplankton, crustacean or mollusk,

and fish species chosen from among the most sensitive species documented in the scientific literature or accepted by EPA as being reliable test organisms to determine the anticipated impact of the wastes on the ecosystem at the disposal site. Bioassays, except on phytoplankton or zooplankton, shall be run for a minimum of 96 hours under temperature, salinity, and dissolved oxygen conditions representing the extremes of environmental stress at the disposal site. Bioassays on phytoplankton or zooplankton may be run for shorter periods of time as appropriate for the organisms tested at the discretion of EPA, or EPA and the Corps of Engineers, as the case may be.

(d) "Appropriate sensitive benthic marine organisms" means at least one species each representing filter-feeding, deposit-feeding, and burrowing species chosen from among the most sensitive species accepted by EPA as being reliable test organisms to determine the anticipated impact on the site; provided, however, that until sufficient species are adequately tested and documented, interim guidance on appropriate organisms available for use will be provided by the Administrator, Regional Administrator, or the District Engineer, as the case may be.

§ Section 227.13(c) Concluded

. . .(3) Bioassays on the suspended particulate and solid phases show that it can be discharged so as not to exceed the limiting permissible concentration as defined in paragraph (b) of Section 227.27.

(d) For the purposes of paragraph (c)(2), major constituents to be analyzed in the liquid phase are those deemed critical by the District Engineer, after evaluating and considering any comments received from the Regional Administrator, and considering known sources of discharges in the area.

APPENDIX B: DREDGED MATERIAL SAMPLE COLLECTION AND PREPARATION

Introduction

1. The collection and preparation of disposal site water and dredged material samples for testing is one of the more important phases of evaluating the impact of dredged material discharge upon the aquatic environment. Samples that are improperly collected, preserved, or prepared will totally invalidate any testing conducted and will lead to erroneous conclusions regarding the potential impact of the proposed discharge. Meticulous attention must therefore be given to all phases of water and sediment sampling, storage, and preparation. The procedures described herein specify the apparatus and procedures to use for sampling water and dredged material and preparing the water and dredged material for chemical analyses and bioassay procedures.

Sample Collection and Preservation

2. Collection and preservation of dredged material and water samples are discussed in this section. The procedures are designed to minimize sample contamination and alteration of the physical or chemical properties of the samples due to freezing, air oxidation, or drying.

Number of samples

3. The number of sediment and water samples to be taken from the dredging or excavation site for processing must be carefully considered because of the extremely heterogeneous nature of samples of this type. The largest source of variation between dredged material samples taken at a dredging site has been shown to be the vertical and horizontal distribution of the samples.[1] Sediment should therefore be collected from a minimum of three sampling stations within the dredging area. Many dredging projects will require more than this minimum number of samples for proper characterization. The number of samples and their location should be selected cooperatively by the District Engineer and Regional Administrator before sample collection begins. The sampling

stations should be located throughout the area to be dredged and should be selected to characterize obviously contaminated as well as noncontaminated areas. The amount of dredged material or water collected should be limited to the amount that can be used within 2 weeks after sampling.

Apparatus

4. The following items are required for water and dredged material sampling and storage.

a. Noncontaminating sediment grab or core sampler (Smith-McIntyre or Van Veen grab, K. B. corer, etc.).

b. Noncontaminating water sampler (Van Dorn water sampler, etc.).

c. Acid-rinsed linear polyethylene bottles for water samples to be analyzed for metals and nutrients.

d. Solvent-rinsed glass bottles with Teflon-lined screw-type lids for water samples to be analyzed for pesticide materials.

e. Plastic jars or bags for collection of dredged material samples.

f. Ice chests for preservation and shipping of dredged material and water samples.

Water sampling

5. Collection of water samples should be made with appropriate noncontaminating water-sampling devices. Special care must be taken to avoid the introduction of contaminants from the sampling devices and containers. To avoid trace metal contamination, sampling devices should be constructed of plastic materials. Prior to use, the sampling devices and containers should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in 10-percent hydrochloric acid (HCl) for 4 hr, and then thoroughly rinsed with metal-free water. Water samples taken for trace organic analyses should be taken with glass or stainless steel devices. If plastic devices must be used, they must be cleaned, aged, and characterized as to the material that may leach from them into the samples. The sampling devices should be thoroughly cleaned, following the procedures outlined in the "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental

Samples,"[2] and then rinsed just before using with the same solvent to be used in the analysis, most probably hexane.

6. A representative disposal site water sample is obtained by collecting 1/3 of the sample volume directly below the water surface, 1/3 from mid-depth in the water column, and 1/3 from approximately 1 m above the sediment surface. The portion of the samples to be used for pesticide material analyses must be stored in glass or aluminum containers. Dredging site water should be collected approximately 1 m above the bottom.

7. The samples should be stored immediately at 2 to 4°C, never frozen. The storage period should be as short as possible to minimize changes in the characteristics of the water. It is recommended that samples be processed within two weeks of collection.

Sediment sampling

8. Sediment samples should be taken with a corer or a grab sampler in a manner designed to ensure that their characteristics are representative of the proposed dredging site. Sampling stations should include known or suspected areas of high contamination as well as more representative areas. The larger the proposed dredging site, the more samples will be required for adequate coverage and characterization. The samples should be placed in airtight linear polyethylene containers. If organic materials are of primary concern, airtight glass storage containers should be used. Care should be taken to ensure that the containers are completely filled by the samples and that air bubbles are not trapped in the containers. The samples should be stored immediately at 2 to 4°C. The samples must never be frozen or dried. The storage period should be as short as possible to minimize changes in the characteristics of the dredged material. It is recommended that the samples be processed within two weeks of collection.

Liquid Phase Sample Preparation

9. Water and liquid phase samples should be prepared for bioassays and/or chemical analysis as soon as possible after collection. The

liquid phase may be prepared with dredging site water for use in chemical analyses as given in Appendix C or with disposal site water for bioassay testing as detailed in Appendices D and E. The volume of solution needed for chemical analyses will vary depending upon the number and type of analyses to be conducted (Appendix C). Appendices D and E should be consulted to determine the volumes required for bioassays.

Apparatus

10. The following items are required.

    a. Laboratory shaker capable of shaking 2-ℓ flasks at approximately 100 excursion per min. Box-type or wrist-action shakers are acceptable.

    b. Several 1-ℓ (or larger) graduated cylinders.

    c. Large (15 cm) powder funnels.

    d. Several 2-ℓ large-mouth graduated Erlenmeyer flasks.

    e. Vacuum or pressure filtration equipment, including vacuum pump or compressed air source and an appropriate filter holder capable of accomodating 47-, 105-, or 155-mm-diam filters.

    f. Presoaked filters with a 0.45-μ pore-size diameter.

    g. Centrifuge capable of handling six 1.0- or 0.5-ℓ centrifuge bottles and operating at 3000 to 5000 rpm.

    h. Plastic sample bottles, 500-ml capacity for storage of water and liquid phase samples for metal and nutrient analyses.

    i. Wide-mouth, 1-gal capacity glass jars with Teflon-lined screw-type lids should be used for sample containers when samples are to be analyzed for pesticide materials. (It may be necessary to purchase jars and Teflon sheets separately; in which case, the Teflon lid liners may be prepared by the laboratory personnel.)

11. Prior to use, all glassware, filtration equipment, and filters should be thoroughly cleaned. Wash all glassware with detergent, rinse five times with tap water, place in a clean 10-percent (or stronger) HCl acid bath for a minimum of 4 hr, rinse five times with tap water, and then rinse five times with distilled or deionized water. Soak filters for a minimum of 2 hr in a 5-M HCl bath and then rinse 10 times with distilled water. It is also a good practice to discard the first 50 ml

of water or liquid phase filtered. Wash all glassware to be used in preparation and analysis of pesticide residues using the eight-step procedure given in the "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples."[2] Flush the glassware just before using with the same solvent to be used in the pesticide analyses.

Sample preparation

12. In order to properly prepare liquid phase samples for chemical analyses or for use in bioassays, the stepwise procedure given below must be followed. For procedural reasons, liquid phase for chemical analyses should be prepared with dredging site water, and liquid phase for bioassays should be prepared with disposal site water.

13. Step 1. Subsample approximately 1 ℓ of sediment from the well-mixed original sample. Mix the sediment and unfiltered disposal site or dredging site water in a volumetric sediment-to-water ratio of 1:4 at room temperature (22 ± 2°C). This is best done by the method of volumetric displacement. One hundred ml of unfiltered water is placed into a graduated Erlenmeyer flask. The sediment subsample is then carefully added via a powder funnel to obtain a total volume of 300 ml. (A 200-ml volume of sediment will now be in the flask.) The flask is then filled to the 1000-ml mark with unfiltered water, which produces a slurry with a final ratio of one volume sediment to four volumes water. If the volume of liquid phase required for bioassay or analysis exceeds 700 to 800 ml, the initial volumes should be proportionately increased (e.g., mix 400 ml of sediment and 1600 ml of water). Alternately, several 1-ℓ dredged material/water slurries may be prepared as outlined above and the filtrates combined to provide sufficient liquid phase.

14. Step 2.

a. Insert an air-diffuser tube almost to the bottom of the flask. Compressed air should be passed through a deionized water trap and then through the diffuser tube and the slurry. The flow rate should be adjusted to agitate the mixture vigorously for 30 min. In addition, the flasks should be stirred manually at 10-min intervals to ensure complete mixing.

b. If it is known that anoxic conditions (zero dissolved

oxygen) will occur at the disposal site or if reproducibility of liquid phase analyses is not a potential problem, the mixing may be accomplished by shaking. During shaking, the oxygen demand of the dredged material may cause the dissolved oxygen concentration in the flask to be reduced to zero. This change can alter the release of chemical contaminants from dredged material to the water and reduce the reproducibility of the liquid phase tests.[3] Shaking is accomplished by capping the flask tightly with a noncontaminating stopper and shaking vigorously on an automatic shaker at about 100 oscillations per min for 30 min. A polyfilm-covered rubber stopper is acceptable for minimum contamination.

15. Step 3. After mixing with air or shaking, allow the suspension to settle for 1 hr.

16. Step 4.

a. If analysis of pesticide or polychlorinated biphenyl (PCB) materials is desired, carefully decant an appropriate portion of the supernatant after the settling period. Samples to be analyzed for pesticide or PCB materials must be free of particulates but should not be filtered, due to the tendency for these materials to adsorb on the filter. However, particulate matter can be removed before analysis by high-speed centrifugation at 10,000 times gravity using Teflon, glass, or aluminum centrifuge tubes.[4]

b. If analyses for nonpesticide or non-PCB materials are desired or if liquid phase bioassays are to be conducted, at the end of the settling period, carefully decant the supernatant into appropriate centrifuge bottles and then centrifuge. The time and rpm's during centrifugation should be selected to reduce the suspended solids concentration substantially and therefore shorten the final filtration process. After centrifugation, vacuum or pressure filter approximately 50 ml of sample through a 0.45-μ filter and discard the filtrate. Filter the remainder of the sample to give a clear final solution.

17. Step 5.

a. Samples to be analyzed for pesticide or PCB materials should immediately undergo solvent extraction, as described in the analytical references given in paragraph 3 Appendix C. The extract may then be held in clean uncontaminating containers for periods up to three or four weeks at -15 to -20°C before the analyses are performed.

b. Samples for metals analysis should be preserved immediately after filtration by lowering the pH to <2 with

3 to 5 ml of concentrated nitric acid per litre.[5] High purity acid, either purchased commercially or prepared by a subboiling unit, must be used.

c. Nutrient analyses should be conducted immediately. Acidification with H_2SO_4 to pH <2 and storage at 4°C may allow the sample to be held for a maximum of 24 hr for ammonia nitrogen, Kjeldahl nitrogen, and nitrate nitrogen analyses.[5] Storage at 4°C will allow holding of samples to be analyzed for dissolved orthophosphate and total dissolved phosphorus for up to 24 hr.[5] Subsamples to be analyzed for cyanide should be preserved with 2 ml of 10 N sodium hydroxide per litre of sample (pH > 12).[5]

d. Liquid phase samples to be used in bioassays must not be preserved or stored. Bioassays should begin as soon as the liquid phase is prepared.

Disposal Site Water Sample Preparation

18. Disposal site water samples are prepared by following the filtration and preservation steps discussed in paragraphs 11, 16, and 17.

Suspended Particulate Phase Sample Preparation

19. The suspended particulate phase, which is used exclusively for bioassays, is prepared in a manner very similar to that for the liquid phase. The steps given in paragraphs 11, 13, 14, and 15 are followed exactly. The suspended particulate phase is the liquid and that material remaining in suspension after the 1-hr settling period (in other words, the suspended particulate phase is an unfiltered liquid phase). With some very fine-grained sediments, it may be necessary to centrifuge the supernatant after the 1-hr settling period. This centrifugation, if used at all, should be only enough to make the test organisms visible during the bioassay. The suspended particulate phase bioassay should begin as soon as the suspended particulate phase is prepared.

Solid Phase Sample Preparation

20. The solid phase of dredged material is used exclusively in bioassays or bioaccumulation studies as discussed in Appendices F and G. The solid phase is defined for bioassessment purposes as sediments of in situ density collected within the dredging site. These sediment samples should be collected and stored as described in paragraph 8. The solid phase for use in bioassays should be prepared immediately prior to beginning the bioassays. Indeed, the solid phase preparation is an integral part of the bioassay procedure and is described in detail in Appendix F, "Guidance for Performing Solid Phase Bioassays."

REFERENCES

1. Brannon, J. M., et al., "Selective Analytical Partitioning of Sediments to Evaluate Potential Mobility of Chemical Constituents During Dredging and Disposal Operations," Technical Report No. D-76-7, December 1976, Environmental Effects Laboratory, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Mississippi.

2. U. S. Environmental Protection Agency, "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples," 1974, Environmental Toxicology Division, Research Triangle Park, North Carolina.

3. Lee, G. F., et al., "Research Study for the Development of Dredged Material Disposal Criteria," Contract Report No. D-75-4, November 1975, prepared by the Institute for Environmental Sciences, University of Texas at Dallas, under contract to the U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Mississippi.

4. Fulk, R., et al., "Laboratory Study of the Release of Pesticide and PCB Materials to the Water Column During Dredging and Disposal Operations," Contract Report No. D-75-6, December 1975, prepared by Envirex, Inc., Milwaukee, Wisconsin, under contract to the U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Mississippi.

5. U. S. Environmental Protection Agency, "Methods for Chemical Analysis of Water and Wastes," EPA-625-/6-74-003, EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Available from the Office of Technology Transfer, Washington, DC 20460).

APPENDIX C: LIQUID PHASE CHEMICAL ANALYSES

1. Presented herein are procedural references for chemical analyses of disposal site water and the liquid phase of dredged material. Samples must be collected, preserved, and prepared according to directions in Appendix B. Trace metal analyses in the liquid phase of dredged material from saline waters are both difficult and complicated because of the high salt content. Special analytical methods such as solvent extraction prior to metal analyses are often required. Also, because of the comparatively low background concentrations of some constituents in samples of this type, the number of replicate analyses of composite liquid phase or disposal site water samples must be carefully considered.

Apparatus

2. The specific equipment necessary for liquid phase chemical analyses will vary depending on the chemical constituent(s) to be analyzed. Referenced procedure manuals should be consulted to determine specific needs, sample size, proper cleanup procedures for glassware and other apparatus, and possible interferences in the analysis.[1, 2, 3]

Procedures

3. The liquid phase may be treated as a water sample and analyzed as described in the referenced methods. Standard procedures for analysis for specific constituents other than pesticides and polychlorinated biphenyl (PCB) materials are given in Table C1 and analytical procedures for pesticides and PCB materials are given in Table C2.

4. Prepare and analyze the liquid phase in triplicate and report the average concentration of the three replicates as the concentration of the contaminant of concern in the liquid phase. Report all concentrations in milligrams or micrograms per litre.

Interpretation of Results

5. Paragraph 227.29(a)(1) of the Register defines the limiting permissible concentration (LPC) of the liquid phase as that concentration

Table C1

Procedural References for Liquid Phase Analytical Methods for Specific Constituents Other than Pesticides and PCBs

Parameter	Reference 1	Reference 2	Reference 3	Other References
		Inorganic Constituents		
Cyanide (total)	p. 40	Method 413 p. 361	Method D2036 p. 503	-
Flouride (total)	p. 59	Method 414A and C p. 389	Method D1179 p. 310	-
Metals (dissolved)	p. 82(4.1.3)	-	-	-
Antimony	p. 94	-	-	-
Arsenic	p. 95	-	-	-
Beryllium	p. 99	-	-	-
Cadmium	p. 101	p. 151	p. 351	-
Chromium	p. 105	p. 151	p. 351	-
Cobalt	p. 107	p. 151	p. 351	-
Copper	p. 108	-	p. 351	-
Iron	p. 110	p. 151	p. 351	-
Lead	p. 112	p. 151	p. 351	-
Manganese	p. 116	p. 151	p. 351	-
Mercury	p. 118, 138	-	p. 344	-
Nickel	p. 141	-	p. 351	-
Selenium	p. 145	-	-	-
Vanadium	p. 153	-	-	-
Zinc	p. 155	p. 151	p. 351	-
Nitrogen				
Ammonia	p. 159	-	-	-
Nitrate-Nitrite	p. 201	Method 419C p. 423	-	-
Total Kjeldahl	p. 175	Method 421 p. 437	-	-
Phosphorus				
Total	p. 249	Method 425C III p. 474	Method D515 p. 387	-
Ortho	p. 249	Method 425F p. 481	Method D515 p. 389	-
		Organic Constituents (except chlorinated hydrocarbons)		
Amines	-	-	-	Ref. 4
Benzidine	-	-	-	Ref. 5 Step 8.2
Methyl mercury	-	-	-	Ref. 6
Oil and grease	p. 229 Step 7.3	-	-	-
Petroleum hydrocarbons	p. 226 Step 6.3	-	-	-
Phenol	p. 241	Method 510 p. 574	Method D1783 p. 542	-
Phenols (specific)	-	-	Method D2580 p. 548 Step 13.1	-

Table C2

Procedural References for Liquid Phase Analytical Methods for Pesticides and PCB Materials

Parameter	Reference 7	Other References
PCBs	Section 10, A	Reference 8
Pesticides:		
N-Aryl carbamates barban chloropropham diuron linuron monuron	-	Reference 9, Step 9.3
O-Aryl carbamates baygon carbaryl (sevin) metacil mesural zectran	-	Reference 10, Step 9.3
Organochlorine aldrin DDT dieldrin endrin toxaphene	Section 10, A	Reference 11, Step 9.3
Organophosphorus malathion methyl parathion parathion guthion demeton diazinon disyston	Section 10, A	Reference 12, Step 9.3
Phenoxy acids 2,4-D silvex 2,4,5-T	-	Reference 13
Triazines altrazine propazine	-	Reference 14, Step 9.3

at which none of the constituents of concern will exceed their respective water-quality criteria after allowance for initial mixing. Whether the LPC would be exceeded can be determined by comparing the volume of the initial mixing zone to the volume of water required to dilute the liquid phase sufficiently to meet the water-quality criteria for the constituent of concern. The appropriate calculations are illustrated in paragraphs 15 through 20 of Appendix H, "Estimation of Initial Mixing." The LPC would be exceeded only if the required dilution volume exceeds the volume of the initial mixing zone.

REFERENCES

1. U. S. Environmental Protection Agency, "Methods for Chemical Analysis of Water and Wastes," EPA-625/6-74-003, July 1974, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Available from the Office of Technology Transfer, Washington, DC 20460.)

2. Rand, M. C., Greenberg, Arnold E., and Taras, Michael J., Editorial Board, Standard Methods for the Examination of Water and Wastewater, 14 ed., 1975, prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

3. American Society for Testing and Materials, 1974 Annual Book of ASTM Standards, Part 31, Water, Philadelphia, Pennsylvania.

4. Anonymous, "Direct Aqueous Injection for Quantification," Analytical Chemistry, Vol. 46, 1974, p. 977. *(Column packing is available through Supelco, Inc., Bellefonte, PA. Request Bulletin 738-Carbopack.)*

5. U. S. Environmental Protection Agency, "Method for Benzidine and Its Salts in Wastewater," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

6. U. S. Environmental Protection Agency, "Methyl Mercury in Sediment," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

7. U. S. Environmental Protection Agency, "Analysis of Pesticide Residues in Human and Environmental Samples," 1974, Environmental Toxicology Division, Research Triangle Park, North Carolina.

8. U. S. Environmental Protection Agency, "Method for Polchlorinated Biphenyls (PCB's) in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

9. U. S. Environmental Protection Agency, "Method for N-Aryl Carbamate and Urea Pesticides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

10. U. S. Environmental Protection Agency, "Method for O-Aryl Carbamate Pesticides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

11. U. S. Environmental Protection Agency, "Method for Organochlorine Pesticides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

12. U. S. Environmental Protection Agency, "Method for Organophosphorus Pesticides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

13. U. S. Environmental Protection Agency, "Method for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

14. U. S. Environmental Protection Agency, "Method for Triazine Pesticides in Industrial Effluents," 1973, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

APPENDIX D: GUIDANCE FOR PERFORMING LIQUID PHASE AND SUSPENDED PARTICULATE PHASE ANIMAL BIOASSAYS

Introduction

1. The described bioassay of appropriate sensitive marine organisms can be used as an aid in evaluating the importance of dissolved chemical constituents released from the sediment during disposal operations. This procedure can also be used to evaluate the effect of suspended particulate matter that is present in the water column for certain periods of time during disposal of dredged material. A series of experimental treatments and controls are established using the liquid phase or suspended particulate phase of the dredged material and disposal site water. The test organisms are added to the test chambers and incubated under standard conditions for a prescribed period of time. The surviving organisms are examined at appropriate intervals to determine if the test material is producing an effect. Phytoplankton bioassays require a somewhat different approach and are described in Appendix E, "Guidance for Performing Liquid Phase and Suspended Particulate Phase Phytoplankton Bioassays."

Apparatus

2. The following items are required for each separate test series, which consists of one set of control and test aquaria with three replicates of each. Appropriate additional items will be needed for each additional test series.

a. Twelve crystallizing dishes (100 mm × 50 mm) to be used as test containers for zooplankton and larvae.

b. Covers for the crystallizing dishes. Sheets of window glass or clear plastic are suitable.

c. Twelve 10-gallon (37.8-ℓ) all-glass aquaria, 26 cm wide, 51 cm long, and 31 cm deep, to be used as test containers for crustaceans, molluscs, and fish.

d. Transfer pipettes with a 0.2- to 0.3-mm bore size and rubber bulbs; transfer pipettes with 7- to 9-mm bore size.

e. Fine-mesh dip nets.

f. Facility for maintaining constant temperature and appropriate photoperiod in the test containers. Any incubator that allows control of the temperature within $\pm$ 1°C and provides acceptable lighting will suffice. Cool-white flourescent lighting located above the test units at a distance of approximately 0.5 to 1 m should be used.

g. A light box with illumination from below for ease in counting zooplankton and larvae.

Species Selection

3. Liquid phase and suspended particulate phase bioassays must utilize appropriate sensitive marine organisms as described in paragraph 227.27(c) of the Register (see Appendix A). The sensitivity of all bioassays is dependent primarily on the selection of appropriate species.

4. If at all possible the test species should be collected from a reference area near the disposal site and similar to it in water quality and substrate sedimentology, but with no recent history of disposal. They should be the same species or be closely related to those species that naturally dominate biological assemblages in the vicinity of the disposal site in the season of the proposed operation. Experience has shown that with reasonable care it is possible to collect test organisms from wild populations and maintain them under controlled conditions with low mortality. However, a preliminary study of the ability of field-collected test organisms to acclimate to laboratory conditions is highly desirable.

5. If it is not practical to use the dominant species collected from near the disposal site, test species may be selected from Table D1 if they are chosen so that, insofar as possible, they are related phylogenetically and/or by ecological requirements to the dominant appropriate sensitive marine organisms expected in the area of the disposal site at the time of the proposed operation. Commercially important organisms from the vicinity of the disposal area may also be included if desired. In Table D1, species are not listed in order of

Table D1

Recommended Appropriate Sensitive Marine Organisms for Use in Liquid Phase and Suspended Particulate Phase Bioassays*

Zooplankton	Crustacean or Mollusc	Fish
Copepods, *Acartia* sp.	Mysid shrimp, *Mysidopsis* sp.** *Neomysis* sp**	Group I
Larvae of recommended crustacean or mollusc species	Grass shrimp, *Palaemonetes* sp. *Palaemon* sp.	Silversides, *Menidia* sp.
	Commercial shrimp, *Penaeus* sp.	Pinfish, *Lagodon rhombiodes*
	Sand shrimp, *Crangon* sp.	Spot, *Leiostomus xanthurus*
	Oceanic shrimp, *Pandalus* sp.	Shiner perch, *Cymatogaster aggregata*
	American lobster, *Homarus americanus*	Group II
	Blue crab, *Callinectes sapidus*	English sole, *Parophrys vetulus*
	Cancer crab, *Cancer* sp.	Flounder, *Platichthys* sp. *Paralichthys* sp. *Limanda* sp.
	Amphipods, *Ampelisca* sp. *Paraphoxus* sp.	Group III
	Cumacean, *Diastylopis* sp.	Sheepshead minnow, *Cyprinodon variegatus*
	Macoma clam, *Macoma* sp.	Mummichog, *Fundulus heteroclitus*
	Nucula clam, *Nucula* sp.	Killifish, *Fundulus* sp.
	Yoldia clam, *Yoldia* sp.	
	Surf clam, *Spisula solidissima*	
	Hard clam (quahog), *Mercenaria* sp.	
	Ocean quahog, *Arctica islandica*	
	Scallop, *Argopectin* sp. *Aequipectin* sp.	
	Gemma clam, *Gemma gemma*	
	Edible mussel, *Mytilus edulis*	

* Lists are not in order of preference or desirability except for the groups of fish.

** All liquid phase and suspended particulate phase bioassays should include one of these species.

preference, except that the fish of group I are the most desirable for bioassay purposes, and those in group III are considered generally less likely to be sensitive indicators of potential effects.

6. All liquid phase and suspended particulate phase bioassays should include a species of mysid shrimp of the genus *Mysidopsis* or *Neomysis*. This will provide an internal standard in all bioassays and form a basis for quality assurance in the regulatory program.

7. It is recommended that juvenile forms, particularly of fish, be utilized because of their generally greater sensitivity than adults. The wet weight of individual test specimens should not be greater than 3 g. Molluscs generally are relatively resistant to many toxicants and therefore are often undesirable for bioassays, but they are very useful for bioaccumulation studies. If used in bioassays, they should be less than 2 cm long. To avoid predation, it probably will be necessary to conduct the bioassay with potential predator and prey species isolated from each other. For example, fish and zooplankton or larvae must be separated to avoid predation. The identity of all test species must be verified by experienced taxonomists. If the bioassay animals are also to be used in estimating bioaccumulation potential, species selection should consider the factors discussed in paragraphs 5, 6, and 7 of Appendix G, "Guidance on Assessing Bioaccumulation Potential."

8. Whatever the source of the animals, collection and handling should be as gentle as possible. Transportation to the laboratory should be in well-aerated water from the animal collection site in which the animals are held at the temperature and salinity from which they were obtained. Animals from established laboratory populations may, of course, be held indefinitely, but animals collected from the field should be held in the laboratory no more than two weeks before bioassays are begun. During this period they must be gradually acclimated to the salinity and temperature at which the bioassay will be conducted. Acclimation from one seasonal extreme to the other should be avoided. Methods for collecting, handling, acclimating, and sizing bioassay organisms given in "Bioassay Procedures for the Ocean Disposal Program"[1] and "Standard Methods for the Examination of Water and

Wastewater"[2] should be followed in all matters for which no guidance is given here.

Sample Collection and Preservation

9. Sediment and water samples are collected and stored and the liquid phase and suspended particulate phase are prepared as described in Appendix B, "Dredged Material Sample Collection and Preparation." Water collected from the disposal site should be used if at all possible. Otherwise uncontaminated seawater or an artificial sea salts mixture (such as that given on page 32 of Reference 1) of the proper salinity may be used.

Experimental Conditions

10. Liquid and suspended particulate phase bioassays should be conducted at a salinity near that expected at the disposal site at the time of the proposed operation. Experimental temperature should be held stable within $\pm$ 2°C of a temperature approximating that expected at the disposal site in the season of the proposed operation. Recommended experimental temperatures are given on a seasonal basis for various zoogeographic areas in the following tabulation.

Summer	Winter	CE Division	EPA Region
20	5	New England, North Atlantic	I, II*, III
25	12	South Atlantic, Lower Mississippi Valley, Southwestern	IV, VI
10	10	North Pacific, South Pacific	IX**, X
25	25	Pacific Ocean	IX**

* Puerto Rico and Virgin Islands are in EPA Region II, but should use temperatures recommended for Region IV.

** Mainland portions of Region IX should use South Pacific Division temperatures; Pacific island portions of Region IX should use Pacific Ocean Division temperatures.

11. Dissolved oxygen should not be allowed to fall below 4 ppm, unless there is reason to believe that depression to lower levels would occur for a substantial period of time in the field during the proposed disposal operation or if lower levels occur naturally at the site. Light intensity should be approximately 1200 $\mu w/cm^2$ using cool-white flourescent bulbs with a 14-hr light and 10-hr dark cycle. The temperature, salinity, dissolved oxygen, and pH in the test containers should be measured and reported daily.

Experimental Procedure

12. Glassware must be extremely clean. Wash all glassware with detergent, rinse five times with tap water, place in a clean 10-percent HCl acid bath for a minimum of 4 hr, rinse five times with tap water, and then rinse five times with distilled water.

13. Establish treatment levels using disposal site water and liquid phase or suspended particulate phase of the material, prepared as described in Appendix B. A minimum of three replicates of each experimental and control condition must be used. More replicates should be used whenever possible, as this will increase the sensitivity and reliability of the test. The final liquid volume in each dish is 200 ml and in each aquarium is 30 ℓ.

14. The following concentrations of the test material are recommended as a minimum, with more being desirable whenever possible.

Percent Liquid Phase	Percent Disposal Site Water
10	90
50	50
100	0

Percent Suspended Particulate Phase	Percent Disposal Site Water
10	90
50	50
100	0

The following controls should be used for each phase:

a. 100-percent fresh culture water of the type in which the animals have been held prior to testing.

b. If the bioassays are conducted with disposal site water, it is advisable to establish an additional control of 100-percent disposal site water.

15. Ten organisms are exposed in each test dish or aquarium. Individual organisms must be randomly assigned to treatments. Make every attempt to collect animals of approximately equal size. Use a pipette to transfer zooplankton and larvae from the laboratory culture vessel to the test containers. Care must be taken during the transfer process to ensure that air is not trapped on the zooplankton and larvae. Place the pipette under the surface of the liquid in the test container and gently release the liquid and animal into the test solution. Juvenile and adult crustaceans, molluscs, and fish are transferred to the test containers in fine-mesh nets. Submerge the net in the test container and gently evert it, releasing the animals. During this process, transfer the minimum amount of culture medium possible with each animal and use a different pipette or net for each concentration of test solution. The utmost care should be taken when transferring any of the animals from holding facilities to the exposure containers to avoid damaging the organisms. Discard any animals that are dropped or physically abused during the transfer. Never touch animals by hand. Reference 1 and 2 provide detailed instructions on handling and transfer procedures.

16. Cover the dishes and incubate the test containers in an appropriate test chamber. Positioning of the test containers holding various concentrations of test solution must be randomized. The test medium is not replaced during the 96-hr experimental period. No aeration is supplied (unless indicated by the considerations in paragraph 11), and the test medium is not stirred. Therefore, some sedimentation will take place during suspended particulate bioassays, and at the end of the test only very fine particles will remain in suspension.

17. Observations should be made at 0, 4, 8, 24, 48, 72, and 96 hr. Animals are counted visually at each observation time with the aid of a light box or dissecting microscope if necessary. Take care to minimize stresses on the animals during counting. Counting should be done

quickly and the animals immediately returned to the test containers. Death is the end point, so the number of living organisms is recorded. Death is determined by lack of movement after a gentle swirl of the dish or gentle touching of a sensitive part with a probe. All crustaceans, both larval and adult, molt at regular intervals, shedding a complete exoskeleton. Care should be taken not to count an exoskeleton as a dead animal. Dead animals may decompose or be eaten between observations. Therefore, always count living, not dead animals. Remove dead organisms and molted exoskeletons at each observation with a pipette or forceps. Care must be taken not to disturb living organisms and to minimize the amount of liquid withdrawn.

Data Analysis and Interpretation

18. The criteria require that liquid and suspended particulate phase bioassay results be interpreted in view of the mixing and dilution expected at the disposal site. According to Section 227.13 of the Register, dredged material can be considered environmentally acceptable for ocean disposal only if bioassay results and initial mixing estimates indicate that the limiting permissible concentration (LPC) will not be exceeded (Section 227.27). Therefore, bioassay results cannot be interpreted until initial mixing calculations are performed, as described in Appendix H, "Estimation of Initial Mixing." Procedures in this section apply to both liquid phase and suspended particulate phase bioassays of all appropriate sensitive marine organisms.

Data presentation

19. Complete survival data in all test containers at each observation time should be presented as shown in Table D2. The species must be identified by scientific name. If greater than 10-percent mortality occurs in the controls, all data must be discarded and the experiment repeated. Control mortalities of 20 percent may be acceptable in zooplankton and larval bioassays. Unacceptably high control mortality indicates the presence of important stresses on the organisms other than the material being tested, such as injury or disease, stressful

Table D2

Hypothetical Results of a Bioassay of the Liquid Phase or Suspended Particulate Phase of a Dredged Material*

Exposure Condition	Replicate	Time of Observation - Number of Survivors						
		0 hr	4 hr	8 hr	24 hr	48 hr	72 hr	96 hr
Culture water control	1	10	10	10	10	10	10	10
	2	10	10	10	8	8	8	8
	3	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{28}$	$\frac{10}{28}$	$\frac{10}{28}$	$\frac{10}{28}$
100-percent test medium	1	10	10	10	8	7	5	4
	2	10	10	9	7	6	4	2
	3	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{29}$	$\frac{8}{23}$	$\frac{6}{19}$	$\frac{4}{13}$	$\frac{3}{9}$
50-percent test medium	1	10	10	10	9	9	7	6
	2	10	10	10	10	9	8	6
	3	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{29}$	$\frac{9}{27}$	$\frac{7}{22}$	$\frac{6}{18}$
10-percent test medium	1	10	10	10	10	9	9	9
	2	10	10	10	9	9	9	9
	3	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{30}$	$\frac{10}{29}$	$\frac{10}{28}$	$\frac{10}{28}$	$\frac{10}{28}$

* Appropriate sensitive marine organisms must be used, and table must include the scientific name of species tested.

physical or chemical conditions in the test containers, or improper handling, acclimation, or feeding. If less than 10-percent (or 20-percent) mortality occurs in the controls, the data may be evaluated.

Statistical analysis

20. To assess the possibility of unacceptable adverse impacts in the water column, it is necessary to statistically compare the 96-hr survival in the appropriate control to survival in the 100-percent test medium and then to determine the LPC. If mortalities are similar in both controls, the culture water control is appropriate for comparison to the 100-percent test medium, Higher mortality in the disposal site water control indicates a potentially adverse effect of the water at the disposal site. In this case the disposal site control is the appropriate one for comparison with the 100-percent test medium in order to estimate what, if any, additional effect might be caused by the proposed disposal.

21. It is possible that the liquid and suspended particulate phases of some dredged materials will cause no mortality, and total survival in the test medium may be equal to or higher than survival in the controls. If so, visual inspection of the data is adequate and no statistical analyses are needed. Such cases have been documented and in no way reflect on the quality of the bioassay, simply indicating an absence of lethal effects of the dredged material. The LPC cannot be precisely specified in such cases because the acutely toxic concentration cannot be determined, but is known to be at least 100-percent test medium concentration. However, if the acutely toxic concentration is assumed to be 100-percent test medium concentration, the LPC could be exceeded only if the calculations from the appropriate part of Appendix H predicted dilution by a factor of 0.01 or less during initial mixing.

22. If survival in the appropriate control is higher than that in the 100-percent test medium after 96 hr, these sets of data must be compared statistically by use of the t-test as illustrated below using the data from Table D2. Before calculation of t, it is necessary to determine whether the variances of the two sets of data are homogeneous. This is determined by Cochran's test for the homogeneity of variances,

in which C is calculated as the ratio of the largest variance to the sum of all the variances.

$$C = \frac{S^2_{max}}{\Sigma S^2} = \frac{1.33}{2.33} = 0.5708 \qquad \text{(D1)}$$

where

S^2_{max} = larger variance of either the control data or the 100-percent test medium data, calculated as in the example of paragraph 25

ΣS^2 = sum of both variances

23. This C-value is evaluated by comparing it to the tabulated C-value given in the table that is Enclosure 1 to this appendix. In the table, k is the number of treatment variances summed in the denominator (2 in this case), and ν is one less than the number of observations contributing to each variance (3 - 1 = 2 in this case). Therefore, the tabulated value of C in this example is 0.9750.

24. If the calculated C-value is smaller than the tabulated C-value, as it is here, the calculated value is not significant at the 95-percent confidence level, and the variances may be considered homogeneous. If the calculated C-value is larger than the tabulated C-value, the variances are not homogeneous. In such cases, all data should be transformed in order to achieve homogeneity of variances. Such transformations are performed on each datum by obtaining either the natural logarithm of (X + 1) or the arcsin $\sqrt{X}$, where X is the datum. In order to use the arcsin $\sqrt{X}$ transformation, the data must be in the form of a percent expressed as a decimal fraction (i.e., 0.80 survival, not 80-percent survival). Recalculate the C-value using data transformed by either of these methods. If variances are now found to be homogeneous, use the transformed data in all t-calculations. If variances are still nonhomogeneous, t is calculated by using the original data, with a different evaluation given in paragraph 27.

25. The t-value for the 96-hr control and 100-percent test medium data in Table D2 is calculated as follows:

Replicate	Number of Survivors: Control	100% test medium
1	10	4
2	8	2
3	10	3
sum of data = ΣX =	28	9
mean $\bar{X} = \frac{\Sigma X}{n}$ =	9.33	3.00
sum of squares $SS = \Sigma(X-\bar{X})^2$ =	2.67	2.00
variance $S^2 = \frac{SS}{n-1}$ =	1.33	1.00

$$t = \frac{\left|\bar{X}_c - \bar{X}_{100}\right|}{\sqrt{\frac{S_c^2 + S_{100}^2}{n}}} = \frac{|9.33 - 3.00|}{\sqrt{\frac{1.33 + 1.00}{3}}} = \frac{6.33}{\sqrt{0.7767}} = 7.18 \qquad \text{(D2)}$$

where

$\left|\bar{X}_c - \bar{X}_{100}\right|$ = absolute value of mean of control minus mean of the 100-percent test medium data

S_c^2 and S_{100}^2 = variances for control and 100-percent test medium data, respectively

n = number of data points in each set

26. This t-value is evaluated by comparing it to the tabulated t-value given in Reference 3 at the 0.05-probability level with the appropriate degrees of freedom (df); in this case, 2(n-1) = 4. It is important that the tabulated $t_{.05}$ value be obtained from a table labeled "one-tailed t values" or "t values, sign considered," or some similar designation. Alternatively, the appropriate t-value may be obtained from a standard table at the 0.1-probability level. In this example, the appropriate t value is:

$$t_{.05(4)} = 2.13$$

Since the calculated t-value is larger than the tabulated t-value, the difference between the control survival and the 100-percent test medium survival is statistically significant at the 95-percent confidence level.

27. When variances of both the original and transformed data are nonhomogeneous, analyze the original data as described above. However, when nonhomogeneous data are analyzed, the calculated t-value must be evaluated by comparing it to the tabulated value for (n-1) = 2 df, instead of 2(n-1) = 4 df. This in effect raises the tabulated t-value, making a difference less likely to be detected.

28. If no statistical difference at the 95-percent confidence level had been shown between survival in the control and test medium, the situation with regard to the LPC would be identical to that described in paragraph 21. If no difference at the 95-percent confidence level is shown between survival in the control and test medium, no effect of the liquid or suspended particulate phase could be predicted for the disposal site, even if no dilution occurred for 96 hr. This obviously will not actually occur at any ocean disposal site. Thus, when no differences are detected between control and test survival after 96 hr, the analysis may be considered complete at this point with no indication of potential impact of the liquid (or suspended particulate) phase if the proposed disposal operation occurs.

29. However, some dredged material may produce data such as the example from Table D2, which showed a statistically significant reduction in survival after 96-hr exposure to 100-percent test medium. In such cases it is necessary to compare bioassay results to the mixing and dilution expected at the disposal site in order to determine whether the LPC would be exceeded. Only then can a prediction be made of the likelihood of adverse effects in the water column if the disposal occurs.

Limiting permissible concentration

30. The likelihood of adverse effects is evaluated by first constructing a time-concentration mortality curve from the bioassay data, which can be compared graphically to the time-concentration relationship for dilution of the material as calculated in Appendix H, "Estimation of Initial Mixing." A time-concentration mortality curve is constructed from the bioassay data by calculating the LC50 (lethal concentration to 50 percent of the sample) for each observation time. This is possible only when 50-percent or greater mortality actually occurs in the highest

concentration of test medium. Thus for the data in Table D2, an LC50 can be calculated for the 72- and 96-hr observations, but not for earlier observations. Calculation of LC50 values can be performed by a variety of methods;[2] where verified computer programs are not available, the method of Litchfield and Wilcoxon[4] is recommended. A sample calculation using the data from Table D2 is given in the following paragraphs.

31. Mortality of at least 50 percent was first observed after 72 hr. The 72-hr LC50 is calculated by arranging the 72-hr experimental data from Table D2 as shown in the first three columns of the following tabulation.

Percent Dose	Dead/ Tested	Percent Dead			Contribution to χ^2
		Observed	Expected	Observed Minus Expected	
10	2/30	6.7	2.0	4.7	0.110
50	8/30	26.7	30.0	3.3	0.005
100	17/30	56.7	57.0	0.3	0.000
					0.115
					× 30
				Overall contribution to χ^2 =	3.45

32. Special so-called "probability paper" is then used to plot observed percent dead on the probability axis against concentration of test medium, as with the closed circles and solid line in Figure D1. A line appearing to fit the data is then drawn through the plotted points. Column 4 of the preceding tabulation is the percent dead "predicted" at the test concentrations by the line just drawn. Column 5 is the absolute value of the difference between columns 3 and 4. Column 6, the contribution of Chi^2 (χ^2), is obtained from Nomograph 1 in the paper by Litchfield and Wilcoxon.[4] The individual contributions to χ^2 are summed and multiplied by the number of animals per dose, 30 in this example, to obtain the overall contribution to χ^2. The "goodness of fit" of the line drawn above to the data is then tested by comparing the calculated overall contribution to χ^2 to the tabulated value at the 95-percent confidence level with n - 2 df. In this case, 3.45 is less than the tabulated value of 3.84 (df = n - 2 = 3 - 2 = 1) in Litchfield and

Figure D1. Graphical presentation of 72-hr and 96-hr mortality data from Table D3 (NOTE: Probability paper must be used.)

Wilcoxon's Table 2.[4] Therefore the line is considered to fit the data adequately. If the calculated value had exceeded the tabulated value, the line would not be considered an acceptable representation of the data, and another line would have to be tried until an acceptable fit was obtained.

33. Once a satisfactory line is obtained, the LC16, LC50, and LC84 values (lethal concentration to the stated percent of the sample) for this observation time are read from the graph. In this case:

LC16 = 30-percent test medium
LC50 = 84-percent test medium
LC84 = 123-percent test medium

34. The slope S of the line is then calculated as:

$$S = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2} = \frac{\frac{123}{84} + \frac{84}{30}}{2} = 2.13 \qquad (D3)$$

35. It is then necessary to determine $\sqrt{N'}$ where N' is the total number of test animals represented by the observed data points falling between 16 and 84 percent expected effects. In this case, $N' = 60$, $\sqrt{N'} = \sqrt{60} = 7.75$.

36. The next step is calculated of FLC50, the factor by which the LC50 is manipulated to obtain the 95-percent confidence limits about the LC50.

$$FLC50 = (S)^{\frac{2.77}{\sqrt{N'}}} = (2.13)^{\frac{2.77}{7.75}} = (2.13)^{.36} = 1.31 \qquad (D4)$$

where 2.77 is constant. The expoential calculation can be solved from Litchfield and Wilcoxon's Nomograph 2.[4] The upper confidence limit (UCL) and lower confidence limit (LCL) about the LC50 at the 95-percent confidence level are then determined as follows:

$$UCL = (LC50) \times (FLC50) = (84\%) \times (1.31) = 110\% \qquad (D5)$$
$$LCL = (LC50) \div (FLC50) = (84\%) \div (1.31) = 64\% \qquad (D6)$$

37. According to these calculations, the estimate of the concentration of test material required to kill 50 percent of the test organisms after 72-hr exposure is 84-percent test medium. That is, the calculated 72-hr LC50 equals 64 percent, and we can say with 95 percent

confidence that the true 72-hr LC50 lies between 64- and 110-percent test medium.

38. The same process is used to calculate the 96-hr LC50 and its confidence limits, as illustrated by the open circles and broken line in Figure D1 and shown in Table D3. According to these calculations,

Table D3

Calculation of 96-hr LC50 for Data from Table D2

Percent Dose	Dead/ Tested	Percent Dead Observed	Percent Dead Expected	Observed minus Expected	Contribution to χ^2
10	2/30	6.7	4.0	2.7	0.019
50	12/30	40.0	42.0	2.0	0.002
100	21/30	70.0	69.0	1.0	0.001
					0.022
					× 30

Overall contribution to χ^2 = 0.66

df = n-2 = 3-2 = 1, tabulated χ^2 = 3.84

LC16 = 22% test medium

LC50 = 60% test medium

LC84 = 117% test medium

$$\text{Slope } S = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2} = \frac{\frac{117}{60} + \frac{60}{22}}{2} = 2.34$$

$$N' = 60, \quad \sqrt{N'} = \sqrt{60} = 7.75$$

$$FLC50 = (S)^{\frac{2.77}{\sqrt{N'}}} = (2.34)^{\frac{2.77}{7.75}} = (2.34)^{.36} = 1.35$$

UCL = (LC50) × (FCL50) = (60%) × (1.35) = 81%

LCL = (LC50) ÷ (FLC50) = (60%) ÷ (1.35) = 44%

the 96-hr LC50 is 60-percent test medium, and we can say with 95 percent confidence that the true 96-hr LC50 lies between 44- and 81-percent test medium.

39. The LC50 estimates and their 95-percent confidence limits for each observation are then plotted against time, as in Figure D2. This illustrates the relationship of concentration and exposure time causing 50-percent mortality in the bioassay and is an estimate of

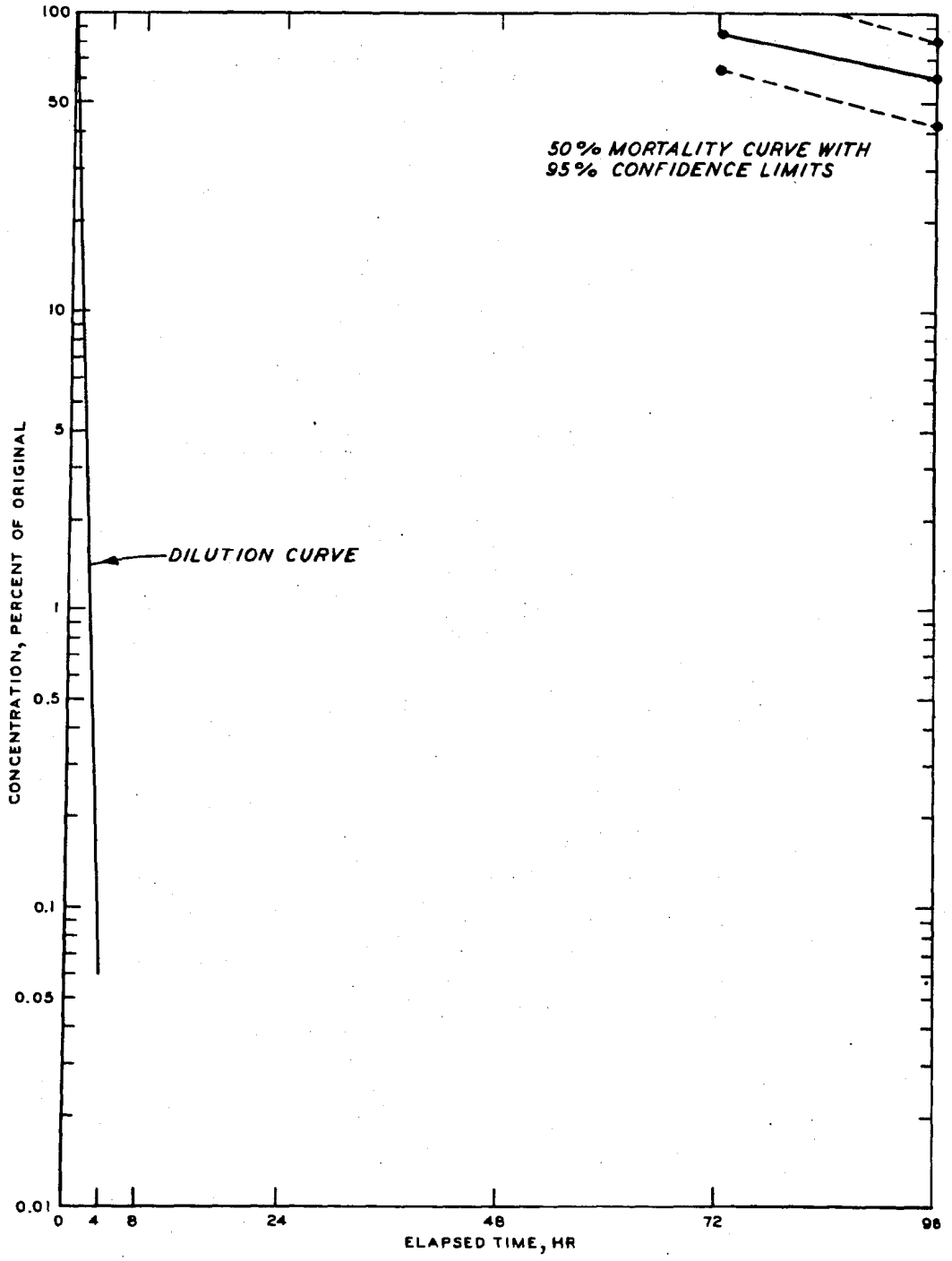

Figure D2. Comparison of time-concentration mortality curve for data from Table D3 with estimated dilution curve

conditions required to produce a similar effect in the field. To determine whether the LPC might be exceeded in the field, this time-concentration mortality curve is graphically compared to the expected dilution curve from Appendix H (Figure D2). The best available mixing estimate, as described in Section 227.29 of the Register and discussed in Appendix H, should be used to derive the time-concentration relationship for dilution to be compared to the time-concentration mortality curve. The initial mixing example used here is for the suspended particulate phase and was taken from Appendix H, paragraphs 24 through 28. It assumes complete lack of knowledge concerning mixing at the disposal site and utilizes a hypothetical disposal operation and the arbitrary mixing calculation of paragraph 227.29(b)(1) of the Register.

40. Paragraphs 227.29(a) and 227.27(a) of the Register state that a concentration of 0.01 (or other factor) of the toxic concentration of the liquid phase shall never be exceeded beyond the boundaries of the disposal site and may be exceeded within the disposal site only during the 4 hr following dumping. The suspended particulate phase is treated similarly, except that the application factor is not included and instead the Register specifies that "unreasonable effects" are forbidden. To help ensure that such effects do not occur and for the sake of uniformity of interpretation, it is recommended that the application factor of 0.01 of the toxic concentration (or other factor as specified in paragraph 227.27(a)(3)) be applied to suspended particulate as well as liquid phase bioassay interpretations.

41. In Figure D2, illustrating the data from Table D2, both the 4-hr and long-term requirements of the LPC are met. After 4 hr, the toxic concentration cannot be precisely specified but is greater than 100 percent of the original suspended particulate phase concentration, and during initial mixing the predicted dilution is by a factor of more than 1600, to 0.06 percent of the original suspended particulate phase concentration. Since the dilution curve and mortality curve continue to diverge, the LPC requirement that a concentration of 0.01 of the toxic concentration shall not be exceeded is met both at the end of and beyond the 4-hr initial mixing period. Therefore, the bioassay would

be considered to have given no indication that the material might produce any environmentally unacceptable impacts in the water column.

42. Figure D3 is a hypothetical case illustrating a situation where the LPC would not be met. It should be emphasized that a situation as severe as this, both in terms of high mortality and low dilution, has never been documented for either the liquid or suspended particulate phase of dredged material. This hypothetical situation is purely for illustrative purposes. In Figure D3, the LPC is exceeded after the 4-hr initial mixing period and at 8 and 24 hr because the concentration predicted by the dilution curve is greater than 0.01 of the lower 95-percent confidence limit about the time-concentration mortality curve. At 48 hr the LPC is satisfied, since the predicted concentration is less than 0.01 of the lower 95-percent confidence limit about the toxic concentration. However, at 72 hr, the LPC is again exceeded. Both the 4-hr and long-term considerations of the LPC must be met to satisfy the criteria. Therefore, this hypothetical situation does not meet the LPC, and if a bioassay gave similar results, it would be considered to have shown the material to have a real potential for causing environmentally unacceptable impacts in the water column.

43. Procedures for using the bioassay animals to estimate the potential for bioaccumulation of contaminants from the liquid or suspended particulate phases of dredged material are discussed in Appendix G, "Guidance for Assessing Bioaccumulation Potential."

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1. U. S. Environmental Protection Agency, "Bioassay Procedures for the Ocean Disposal Permit Program," EPA-600/9-76-010, 1976, EPA Environmental Research Laboratory, Office of Research and Development, Gulf Breeze, Florida.
2. Rand, M. C., Greenberg, Arnold E., and Taras, Michael J., Editorial Board, Standard Methods for the Examination of Water and Wastewater, 14 ed., 1975, prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

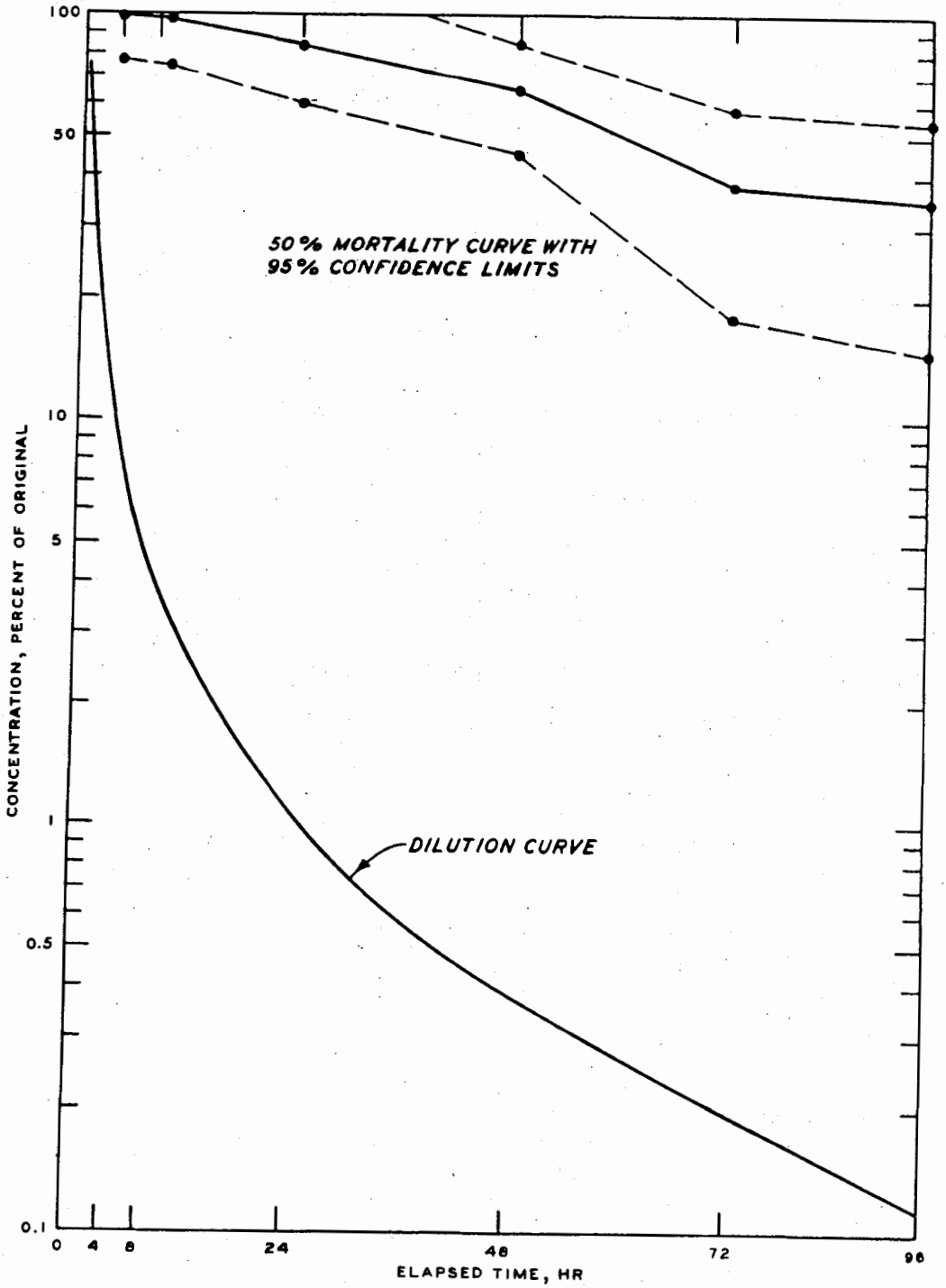

Figure D3. Comparison of *hypothetical* time-concentration mortality curve with *hypothetical* dilution curve

3. Rohlf, F. J., and Sokal, R. R., Statistical Tables, 1969, W. H. Freeman Company, San Francisco, California.

4. Litchfield, J. T., Jr., and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," Journal of Pharmacology and Experimental Therapeutics, Vol. 96, 1949, pp 99-117.

Enclosure 1

Critical Values for Cochran's Test*

Values given are for the statistic (largest s^2)/(Σs_i^2), where each of the k values of s^2 has ν degrees of freedom.

PERCENTILE 95

k \ ν	1	2	3	4	5	6	7	8	9	10	16	36	144	∞
2	0.9985	0.9750	0.9392	0.9057	0.8772	0.8534	0.8332	0.8159	0.8010	0.7880	0.7341	0.6602	0.5813	0.5000
3	0.9669	0.8709	0.7977	0.7457	0.7071	0.6771	0.6530	0.6333	0.6167	0.6025	0.5466	0.4748	0.4031	0.3333
4	0.9065	0.7679	0.6841	0.6287	0.5895	0.5598	0.5365	0.5175	0.5017	0.4884	0.4366	0.3720	0.3093	0.2500
5	0.8412	0.6838	0.5981	0.5441	0.5065	0.4783	0.4564	0.4387	0.4241	0.4118	0.3645	0.3066	0.2513	0.2000
6	0.7808	0.6161	0.5321	0.4803	0.4447	0.4184	0.3980	0.3817	0.3682	0.3568	0.3135	0.2612	0.2119	0.1667
7	0.7271	0.5612	0.4800	0.4307	0.3974	0.3726	0.3535	0.3384	0.3259	0.3154	0.2756	0.2278	0.1833	0.1429
8	0.6798	0.5157	0.4377	0.3910	0.3595	0.3362	0.3185	0.3043	0.2926	0.2829	0.2462	0.2022	0.1616	0.1250
9	0.6385	0.4775	0.4027	0.3584	0.3286	0.3067	0.2901	0.2768	0.2659	0.2568	0.2226	0.1820	0.1446	0.1111
10	0.6020	0.4450	0.3733	0.3311	0.3029	0.2823	0.2666	0.2541	0.2439	0.2353	0.2032	0.1655	0.1308	0.1000
12	0.5410	0.3924	0.3264	0.2880	0.2624	0.2439	0.2299	0.2187	0.2098	0.2020	0.1737	0.1403	0.1100	0.0833
15	0.4709	0.3346	0.2758	0.2419	0.2195	0.2034	0.1911	0.1815	0.1736	0.1671	0.1429	0.1144	0.0889	0.0667
20	0.3894	0.2705	0.2205	0.1921	0.1735	0.1602	0.1501	0.1422	0.1357	0.1303	0.1108	0.0879	0.0675	0.0500
24	0.3434	0.2354	0.1907	0.1656	0.1493	0.1374	0.1286	0.1216	0.1160	0.1113	0.0942	0.0743	0.0567	0.0417
30	0.2929	0.1980	0.1593	0.1377	0.1237	0.1137	0.1061	0.1002	0.0958	0.0921	0.0771	0.0604	0.0457	0.0333
40	0.2370	0.1576	0.1259	0.1082	0.0968	0.0887	0.0827	0.0780	0.0745	0.0713	0.0595	0.0462	0.0347	0.0250
60	0.1737	0.1131	0.0895	0.0765	0.0682	0.0623	0.0583	0.0552	0.0520	0.0497	0.0411	0.0316	0.0234	0.0167
120	0.0998	0.0632	0.0495	0.0419	0.0371	0.0337	0.0312	0.0292	0.0279	0.0266	0.0218	0.0165	0.0120	0.0083
∞	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* By permission from C. Eisenhart, M. W. Hastay, W. A. Wallis, *Techniques of Statistical Analysis*, chap. 15. McGraw-Hill Book Company, New York, 1947.

APPENDIX E: GUIDANCE FOR PERFORMING LIQUID PHASE AND SUSPENDED PARTICULATE PHASE PHYTOPLANKTON BIOASSAYS

Introduction

1. Paragraph 227.27(c) of the Register (see Appendix A) includes "phytoplankton or zooplankton" as one of the three groups of appropriate sensitive marine organisms to be used in bioassays. Phytoplankton bioassays can give information on the potential availability of contaminants associated with the sediment proposed for dredging. However, because of the extremely dynamic and variable nature of normal phytoplankton assemblages and because of the rapid mixing and dilution that takes place in the water column, it is widely felt that effects on phytoplankton are generally of minimal environmental concern at ocean sites for dredged material disposal.

2. Phytoplankton bioassays are of such a nature that the statistical calculation of dose-response data with confidence limits is not practical, making analyses and interpretation of results somewhat uncertain. In addition, phytoplankton bioassays using the suspended particulate phase are extremely difficult to conduct and interpret because of interferences and predation on the test species by indigenous protozoans in the dredged material being tested. The presence of suspended particulates significantly interferes with the determination of response in many cases, leading to a recommendation against attempts to use suspended particulate phase phytoplankton bioassays. For these reasons, unless there is particular concern about effects on phytoplankton by the disposal operation in question, it is recommended that zooplankton, rather than phytoplankton, bioassays be employed to fulfill this requirement of the criteria. This approach would generally provide the most useful information on potential effects of the disposal being evaluated.

3. If the special circumstances of the case warrant a phytoplankton bioassay, it is conducted by establishing a series of treatments and controls using the liquid phase and filtered disposal site water. The

experimental units are then inoculated with test organisms and held under a specified set of test conditions while a sampling program is conducted to determine response.

Apparatus

4. The following items are required:

   a. Thirty 500-ml Erlenmeyer flasks made of Pyrex or Kimex glass.

   b. Plastic beakers or stainless steel caps to cover the 500-ml Erlenmeyer flasks.

   c. Facility for growing algae at constant temperature, illumination, and shaking rate. Any incubator that allows temperature control within $\pm 2^{o}C$, light intensity of approximately 1100 to 1500 $\mu w/cm^2$ using cool-white flourescent bulbs, and a shaking rate of 100 rpm will suffice.

   d. Equipment required for evaluation of response. Requirements will depend on whether cell counts, C^{14} uptake, productivity, or chlorophyll values are the responses to be measured. [1, 2, 3]

Species Selection

5. Phytoplankton should be collected from the disposal site and isolated into axenic cultures for use in the bioassays when this is permitted by practical considerations and the expertise of the experimenter. Otherwise, the species listed in the following tabulation are recommended and may be purchased for laboratory culture as indicated. Methods for collecting and culturing algae are given in "Standard Methods for the Examination of Water and Wastewater,"[1] "Bioassay Procedures for the Ocean Disposal Permit Program,"[2] and "Marine Algal Assay Procedure: Bottle Test."[3]

Species	Source
Skeletonema sp.	USEPA Environmental Research Center Corvallis, Oregon 37330 or

Species	Source
	USEPA Environmental Research Center Narragansett, Rhode Island 02874
Chlorococcum (Milford "C") 819 *Cyclotella* sp. 1269 *Porphyridium* sp. 637	Department of Botany Culture Collection of Algae Indiana University Bloomington, Indiana 41701
Cyclotella sp. 1269	USEPA Environmental Research Center Narragansett, Rhode Island 02874

Sample Collection and Preservation

6. Sediment and water samples are collected and stored and the liquid phase (or suspended particulate phase) is prepared as described in Appendix B, "Dredged Material Sample Collection and Preparation."

Experimental Conditions (Liquid or Suspended Particulate Phase)

7. Procedures for the algal assay for marine disposal sites are similar to those described in Reference 3. This reference gives details of the procedure and rationale and must be used in conjunction with the guidance provided here.

8. Grow stock algal cultures in synthetic nutrient medium.[3] Start new cultures each week by transferring 0.5 ml of a one-week-old culture to 100 ml of fresh medium using aseptic technique. Grow stock cultures at approximately 18-20°C under continuous cool-white floures-cent lighting at an intensity of approximately 1500 $\mu w/cm^2$ and shake continuously at 110 rpm. If shaking tables are not available, swirl all flasks at regular intervals at least twice daily. Acclimation of the stock algal cultures should begin at least two weeks prior to the actual test. Salinity of the test water should be approximately that expected at the disposal site. If test species are not maintained at the proper salinity, they should be transferred to medium of appropriate salinity following procedures for adjusting salinity given in Reference 3. The concentration of nutrients in the growth medium should be reduced

to 20 percent of the stock growth medium during the acclimation period. The algae should also be acclimated to the temperature given in paragraph 10 of Appendix D. The rate of temperature change should not exceed 2°C every 24 hr. Photoperiod should be 14 hr dark and 10 hr light during the acclimation period.

9. Use 500-ml Erlenmeyer flasks covered with beakers or stainless steel caps for culture vessels. Wash all glassware with nonphosphate detergent, rinse with tap water, place in a clean 10-percent HCl acid bath for a minimum of 4 hr, and then rinse five times with distilled water.

Experimental Procedure

10. Establish treatment levels using the liquid or suspended particulate phase, disposal site water, and an inoculum of the test organism to produce a total liquid volume of 100 ml in 500-ml Erlenmeyer flasks when cell counts are the parameter of interest. A greater volume will be required for some of the techniques required for measuring other responses, such as C^{14} uptake or chlorophyll.[1, 2, 3] Establish at least three replicates of each of the following treatment levels and controls:

Percent Liquid Phase	Percent Disposal Site Water
100	0
50	50
10	90

Controls: 100-percent disposal site water
100-percent synthetic algal growth medium

11. Inhibition of growth could be the result of lack of required nutrients or the availability of toxicants. As an aid in determining if toxic chemicals are available to the phytoplankton from the liquid or suspended particulate phase being tested, nutrient additions are useful. Adjust the concentration of a stock solution of growth medium such that when 1 ml is added to the following flasks, the final concentration of the nutrients in each flask is equivalent to 10 percent of

the stock growth medium. The following treatments should receive nutrient additions:

Percent Liquid Phase	Percent Disposal Site Water
100	0
50	50
10	90

Control: 100-percent disposal site water

Also, establish a set of control flasks that contain only 10 percent of the nutrients of the stock growth medium.

12. Prepare the inoculum by centrifuging and washing stock culture cells with sterile artificial seawater of appropriate salinity without nutrients. Adjust the inoculum cell concentration by dilution with sterile seawater; then pipette the inoculum into the test water to give a starting concentration in the test waters of 1000 cells per ml.

13. Distribute the flasks randomly in incubation chambers. Temperature should be set at the level recommended in paragraph 10 of Appendix D ($\pm 2^{o}C$), lighting intensity at approximately 1100 to 1500 $\mu w/cm^2$ using cool-white flourescent bulbs on a 14 hr dark and 10 hr light photoperiod, and the shaking rate at 110 rpm throughout the assays. Test salinity should be approximately that expected at the disposal site. It is important that all test containers are exposed to the same conditions. Continue the assays until the maximum cell number occurs in each treatment level. This does not necessarily occur on the same day for each treatment. Cell numbers can be used to determine cell volume as described in Reference 3 and are a suitable method for reporting results and comparing effects among treatment levels.

14. Determine the effect of the test solution on the algae by comparing the response in the controls to that in the flasks containing the test solution. This may be done by comparing cell counts, cell volume, C^{14} uptake, or chlorophyll values. Procedures for these methods for measuring algal response may be found in Reference 1, 2, and 3. Whatever method of measurement is chosen, observations should be made after 4 hr, 24 hr, and at 24-hr intervals thereafter.

15. The differences measured (e.g., cell counts) can be compared at different times during the bioassay depending upon the type of information needed. The maximum standing crop can be compared when controls and treatments have reached the maximum biomass. This parameter is helpful in predicting total effects in situations where there is concern that frequent use of the disposal site may affect water quality for extended periods of time. Shorter term effects can be compared by calculating the maximum specific growth rate[3] between the controls and experimental treatments. Additional information about potential short-term effects can be gained by comparing the measured parameter at daily intervals during the bioassay. If there is a lag in the initiation of growth, comparing daily measurements will show this and could indicate short-term problems. For example, it is conceivable that the control and experimental cultures will all reach the same maximum biomass, but their rate of attaining that biomass may vary because of differences in the onset of rapid growth or different maximum specific growth rates.

Data Analysis and Interpretation

16. The interpretation of phytoplankton bioassay results must consider mixing and dilution, and it must be determined whether the limiting permissible concentration (LPC) would be exceeded if the proposed disposal occurred. To do so for phytoplankton, it is first necessary to calculate the effective concentration causing 50 percent inhibition (EC50) of the sample relative to the controls, rather than the lethal concentration (LC50).

17. Because phytoplankton data are estimates of population response, rather than discrete counts of individual responses like animal bioassay data, the statistical analyses described for animal bioassays are not applicable to photoplankton data. Therefore, an approximate graphical method is used to estimate the EC50. These estimates are made at each observation time by first expressing the response in each test concentration as a percent of control response. Using semi-logarithmic coordinate paper, graph response as percent of control

response on the arithmetic axis and percent of liquid phase (or suspended particulate phase) concentration on the logarithmic axis. On this graph plot the average response at the test concentration giving somewhat less than 50 percent of the control response and the average response at the test concentration giving somewhat greater than 50 percent of the control response. Zero and 100-percent response should not be used for this purpose. Draw a straight line between the two points. The concentration at which this line crosses the 50-percent response line is an estimate of the EC50 value. This procedure is described in Reference 2 on pages 24-25 and illustrated on page 13.

18. It is possible to estimate an EC50 value only when at least one test condition produces less and one produces more than 50 percent of the control response. In some bioassays less than 50 percent effect may occur in all test conditions. Such cases have been documented and in no way reflect on the quality of the bioassay; the data simply indicates an absence of marked toxic effects of the dredged material. The LPC cannot be precisely specified in such cases because the acutely toxic concentration cannot be determined. However, since the acutely toxic concentration is known to be at least 100-percent test medium concentration, the LPC could be exceeded only if the calculations from the appropriate part of Appendix H predicted dilution by a factor of 0.01 or less during initial mixing.

19. A time-concentration effect curve is constructed from the bioassay data by plotting the EC50 value for each observation against time. This response curve is then compared to the expected dilution curve from Appendix H to determine whether the LPC might be exceeded in the field. The construction and comparison of these curves is described in paragraphs 39 through 41 of Appendix D.

REFERENCES

1. Rand, M. C., Greenberg, A. E., Taras, M. J. (editorial board), Standard Methods for the Examination of Water and Wastewater, 14 ed., 1975, prepared and published jointly by the American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

2. U. S. Environmental Protection Agency, "Bioassay Procedures for the Ocean Disposal Permit Program," EPA-600/9-76-010, 1976, EPA Environmental Research Laboratory, Office of Research and Development, Gulf Breeze, Florida.

3. U. S. Environmental Protection Agency, "Marine Algal Assay Procedure: Bottle Test," 1974, EPA National Environmental Research Center, Corvallis, Oregon.

APPENDIX F: GUIDANCE FOR PERFORMING SOLID PHASE BIOASSAYS

Introduction

1. This bioassay of appropriate sensitive benthic marine organisms can aid in assessing the potential environmental impact of the solid phase of dredged material proposed for ocean disposal and acts as an indirect indicator of chemical toxicity of the sediment. It provides exposure conditions approximating those that would be experienced by animals living near the boundaries of the disposal site. Several benthic species are allowed to establish themselves in an appropriate reference sediment and are then covered with a layer of the dredged material being evaluated. Survival in the dredged material relative to that in the reference sediment control is used as the primary biotic response criterion.

2. The objective is to determine the potential impact of the solid phase on benthos at and beyond the disposal site boundaries. The concept of a disposal site implies that conditions within that site may be adverse, but that conditions beyond its boundaries cannot be. Therefore, this bioassay does not duplicate the depths of sediment deposition that may cover animals directly under the disposal vessel, but rather it approximates the conditions found within or at the disposal site boundaries. The bioassay is designed to determine whether a biological effect is likely, but the bioassay cannot be used to determine the cause of the observed effects. Indeed, if an adverse effect may occur outside the disposal site, it matters little from a regulatory viewpoint whether that effect is due to the physical presence of the sediment or is due to some chemical constituent (s) associated with the sediment carried beyond the site. Therefore, it is important to realize that this benthic bioassay measures the total impact of the dredged material. That impact may be due to an unrecognized pollutant or to the synergistic effects of many pollutants, none of which may have an exceptionally elevated concentration. At the present technical state-of-the-art, it is not possible to determine by any known chemical analysis which pollutant(s) may be the causative agent(s).

Aquarium System

3. The exact dimensions of the test aquaria are not critical, but their bottom area should not be less than 1000 cm^2 nor their volume less than 20 ℓ. Standard 10-gal (37.8-ℓ) all-glass aquaria 26 cm wide, 51 cm long, and 31 cm deep are satisfactory. Five aquaria will be needed for the controls and for each dredged material sampling site tested.

4. Seawater of approximately the same temperature, salinity, and dissolved oxygen as the water near the bottom at the disposal site should be passed through a 20-μ pore size filter and flow into each aquarium at a rate that will replace the aquarium volume at least once every 4 hr. The flow should be directed to achieve good mixing without disturbing a layer of sediment on the aquarium bottom. Water leaves the aquarium through a perforated standpipe covered with a 0.5-mm nylon screen. If a continuous-flow seawater system is not available, animals can be tested in static water aquaria provided that 75 percent of the seawater volume is replaced 1 and 48 hr after the test is begun and at 48-hr intervals thereafter. The frequency of changing should be increased if the control animals appear stressed.

Collection of Sediments and Test Organisms

5. Collect sediments, water, and test species from the field with an appropriate benthic sampler such as the Smith-McIntyre or Van Veen grab. Sediment should be placed in clean nonmetallic containers and maintained at 2 to 4°C from the time of collection until the bioassay begins. Sediment samples must never be frozen or dried. Detailed guidance for collection of sediment and water samples is given in Appendix B. The bioassay must be initiated within two weeks after the sediment and faunal collections. Field-caught test species and the reference sediment must be obtained from an uncontaminated area in the vicinity of the disposal site. This reference sediment must have sedimentological characteristics similar to the disposal site and should be an approximation of the sediment that would be found at the disposal

site if no disposal had ever taken place there. In the likely event that sediment conditions vary substantially within the proposed dredging site, sediment samples from more than one location must be tested. Thus, the bioassay will include at least two and probably more treatments, i.e., the reference substrate control and sediments from one or more locations within the dredging site. Five replicate aquaria are established for each treatment, including the controls.

6. The quantity of sediment needed for the bioassay is dependent on the size of the test aquaria, as discussed in paragraph 3. A 30-mm layer of reference sediment is placed on the bottom of all replicates of all treatments, including the controls. A 15-mm layer of the dredged material in question is placed on top of the 30-mm reference sediment layer in all test replicates, but not the controls. An additional 15-mm layer of the reference sediment is placed on the controls. Sediments for a single treatment should be mixed to ensure homogeneity, and aliquots taken for the bioassay aquaria. If standard 10-gal aquaria are used, a minimum of 4.5 ℓ of reference sediment and 2.5 ℓ of dredged material should be collected for each aquarium.

Species Selection

7. Solid phase bioassays must be conducted with appropriate sensitive benthic marine organisms. Paragraph 227.27(d) of the Register (see Appendix A) defines this to mean at least three species, consisting of one filter-feeding, one deposit-feeding, and one burrowing species. These are broad overlapping general categories and it is recommended that the species be selected to include a crustacean, an infaunal bivalve, and an infaunal polychaete. Infaunal amphipods seem to be among the most sensitive crustacenas and, for this reason, are among the preferred organisms for solid phase bioassays. All solid phase bioassays should include a species of mysid shrimp of the genus *Mysidopsis* or *Neomysis*. This will provide an internal standard in all bioassays and form a basis for quality assurance in the regulatory program.

8. The sensitivity of this and all bioassays is dependent

Table F1

Recommended Appropriate Sensitive Benthic Marine Organisms for Use in Solid Phase Bioassays*

Crustacean	Infaunal Bivalve	Infaunal Polychaete
Mysid shrimp, *Mysidopsis* sp.** (D)	Macoma clam, *Macoma* sp. (F, D)	*Neanthes* sp. (D, B)
Neomysis sp.** (D)	Nucula clam, *Nucula* sp. (F, D)	*Nereis* sp. (B)
Infaunal amphipods,	Surf clam, *Spisula solidissima* (F)	*Nepthys* sp. (B)
Ampelisca sp. (F, D)	Hard clam (quahog), *Mercenaria* sp. (F)	*Glycera* sp. (B, D)
Paraphoxus sp. (F, D, B)	Ocean quahog, *Arctica islandica* (F)	*Urechis* sp. (B, F)
Grass shrimp, *Palaemonetes* sp. (D)	Gemma clam, *Gemma gemma* (F)	*Magelona* sp. (B, D)
Palaemon sp. (D)	Littleneck clam, *Protothaca staminea* (F)	*Owenia* sp. (B, D)
Commercial shrimp, *Panaeus* sp. (D)	Cockle, *Clinocardium nuttali* (F)	*Diopatra* sp. (B, D)
Sand shrimp, *Crangon* sp. (D)		*Glycinde* sp. (B)
Oceanic shrimp, *Pandalus* sp. (D)		
Blue crab, *Callinectes sapidus* (D)		
Cancer crab, *Cancer* sp. (D)		
Cumacean, *Diastylopis* sp. (F, D, B)		
Diastylis sp. (F, D, B)		
Lamprops sp. (F, D, B)		

Note: Parenthetical notations as follows: F - filter-feeding species
D - deposit-feeding species
B - burrowing species

*Lists are not in order of preference or desirability

** All solid phase bioassays should include one of these species

primarily on the selection of appropriate species. If at all possible, the test species should be collected from the area in which the reference substrate is collected. They should be the same species or closely related to those species that naturally dominant benthic assemblages in the vicinity of the disposal site in the season of the proposed operation. Experience has shown that with reasonable care, it is possible to collect test organisms from wild populations and maintain them under control conditions with low mortality. However, a preliminary study of the ability of field-collected test organisms to acclimate to laboratory conditions is highly desirable.

9. If it is not practical to use the dominant species collected from near the disposal site, test species may be selected from Table F1 if they are chosen so that insofar as possible they are related phylogenetically and/or by ecological requirements to the dominant appropriate sensitive benthic marine organisms expected in the area of the disposal site at the time of the proposed operation. Species are not listed in Table F1 in any order of preference or desirability. Commercially important organisms from the vicinity of the disposal site may also be included if desired. The considerations of paragraph 7 must be kept in mind when selecting solid phase bioassay species from any source.

10. Although the Register requires the use of at least three species, the solid phase bioassay example given here uses five species in order to broaden the data base by testing more species of differing sensitivities. As number of test species is increased, it may be necessary to use larger aquaria to avoid overcrowding. Regardless of number of species tested, each should be represented by 20 individuals in each replicate aquarium. It is recommended that juvenile forms, particularly of molluscs and large crustaceans, be utilized because of their generally greater sensitivity than adults. The wet weight of individual test specimens should not be greater than 3 g. Molluscs are often most useful in bioaccumulation studies but should be less than 2 cm long if used in bioassays. To avoid predation, it probably will be necessary to conduct the bioassay with potential predator and prey species isolated from each other. The identity of all test species

should be verified by experienced taxonomists. If the bioassay animals are also to be used in estimating bioaccumulation potential, species selection should consider the factors discussed in paragraphs 5, 6, and 7 of Appendix G, "Guidance for Assessing Bioaccumulation Potential."

11. Test organisms should be collected from the region of the disposal site or cultured in the laboratory. If the organisms are collected from the field with the reference substrate, grab samples for faunal collections should be gently sieved through a 1.0-mm screen, and the animals placed in buckets containing a 2- to 3-cm layer of sediment and several litres of seawater. Whatever the source of the animals, collection and handling should be as rapid and gentle as possible.

12. Transportation to the laboratory should be in well-aerated water from the collection site in which the animals are held at the temperature and salinity from which they were obtained. Benthic animals should be held in the laboratory in aquaria in which approximately 30 mm of reference sediment has been placed. This sediment should contain no other animals and should be from an uncontaminated source similar to the disposal site in sedimentological characteristics. Animals from established laboratory populations may, of course, be held indefinitely, but animals collected from the field should be held in the laboratory for no more than two weeks before bioassays are begun. During this period they must be gradually acclimated, if necessary, to the salinity and temperature at which the bioassay will be conducted. Acclimation of animals from one seasonal extreme to the other should be avoided.

13. Methods for collecting, handling, acclimating, and sizing bioassay organisms given in "Bioassay Procedures for the Ocean Disposal Permit Program,"[1] and "Standard Methods for the Examination of Water and Wastewater."[2] should be followed in all matters for which no guidance is given here.

Experimental Conditions

14. Solid phase bioassays should be conducted at a salinity approximating that expected at the disposal site in the season of the

proposed operation. Water collected from the disposal site should be used if at all possible. Otherwise uncontaminated seawater, or an artificial sea salts mixture such as that given on page 32 of Reference 1, of the proper salintiy may be used. Experimental temperature should be held stable within $\pm 2^{\circ}C$ of a temperature approximating that expected at the disposal site in the season of the proposed operation. Recommended experimental temperatures are given in the following tabulation on a seasonal basis for various zoogeographic areas.

Summer	Winter	CE Division	EPA Region
20	5	New England, North Atlantic	I, II*, III
25	12	South Atlantic, Lower Mississippi Valley, Southwestern	IV, VI
10	10	North Pacific, South Pacific	IX**, X
25	25	Pacific Ocean	IX**

* Puerto Rico and Virgin Islands are in EPA Region II, but should use temperatures recommended for Region IV.

** Mainland portions of Region IX should use South Pacific Division temperatures; Pacific island portions of Region IX should use Pacific Ocean Division temperatures.

Bioassay Procedure

15. The reference substrate, and perhaps the dredged material being tested, may initially contain live organisms of the same species to be used in the bioassay. These must be removed by wet sieving the sediment through a 1.0-mm screen using the smallest amount of seawater possible. The water and sediment must all be retained in a settling container. Place the material retained on the screen in a sorting tray, remove the animals, and return the remainder to the settling container. Allow the sediment to settle for 6 hr, decant the seawater without disturbing the sediment surface, and then mix the sediment to ensure homogeneity. The animal-free dredged material is then returned to its storage containers and held under the conditions specified in paragraph 8 of Appendix B for approximately 48 hr until needed. The animal-free

reference sediment is used at once as described in the following paragraphs. It is recognized that the screening and sedimentation procedure described in this paragraph may result in some alteration of the biological availability of any contaminants present. Although the degree of alteration is unknown, its influence on test results is felt to be minimal. This is the least disruptive method by which the necessary task of removing indigenous animals from the sediments can be accomplished.

16. Partially fill each aquarium with seawater and then add enough reference sediment to produce an even 30-mm layer on the bottom. After 1 hr turn on the seawater and allow it to run for 2 hr before any animals are added. In a static water system, the seawater is replaced after the 30-mm layer of reference sediment has settled for 1 hr, being careful not to resuspend the deposited material.

17. While the reference sediment is settling in the test aquaria, sediments in the animal-holding tanks can be gently siphoned and sieved through a 1.0-mm screen to recapture the test organisms. The utmost care should be taken when handling any of the animals to avoid damaging the organisms. Discard any animals that are dropped or physically abused during the transfer. References 1 and 2 provide instructions on handling and transfer procedures. Specimens of each of the five species are randomly divided among finger bowls equal in number to all aquaria in the bioassay. Each bowl will contain 20 individuals of each species. After the water over the reference sediment has been cleared as described in paragraph 16, the test organisms are released from the bowls to the aquaria and allowed to acclimate for 48 hr. In a static system, 75 percent of the water in the aquaria may be replaced 24 hr after the animals are introduced.

18. During the acclimation period, dead specimens can be removed from the test aquaria and replaced with healthy individuals. It is difficult to determine the exact mortality of infaunal species without disturbing the sediment layer. However, if apparent mortalities exceed 10 percent of the seeded specimens of any species, this test must be discontinued and a new one begun. Species selection, collection, and holding techniques must then be reexamined in an effort to reduce

pretesting mortality in the new test. The bioassay procedure assumes all original animals are alive when the dredged material is introduced, and any undiscovered dead animals in the reference sediment will therefore give a false impression of the effects of the dredged material.

19. After the 48-hr acclimation period, the animals should be established in the reference sediment. The dredged material is divided into aliquots sufficient to produce a 15-mm layer on top of the 30-mm reference sediment layer in the test aquaria. An additional 15-mm layer of reference sediment is placed on the controls. The temperature of the sediment aliquots must be approximately that of the seawater in the aquaria. Turn the water off and remove a seawater volume slightly greater than the dredged material volume to be introduced. Treatments must be randomly assigned to the aquaria. Each bioassay will consist of five aliquots of control sediment and five aliquots from each sampling site within the proposed dredging area. The 15-mm layer is deposited by evenly distributing the sediment aliquot over the water surface. Many sediments can be poured onto the surface if they are mixed with a small volume of seawater. Some crustaceans, such as mysid shrimp, will not survive the physical disruption of the sediment addition and must be placed in the aquaria immediately after the test sediment addition. After allowing 1 hr for settling, the seawater is turned on again. In a static water system, 75 percent of the seawater is replaced 1 and 48 hr after the 15-mm sediment aliquot is added and at 48-hr intervals thereafter.

20. The bioassay continues for 10 days, during which daily records should be kept of obvious mortalities, formation of tubes or burrows, and unusual behavior patterns. Daily levels of salinity, temperature, and dissolved oxygen content of aquaria water should be reported. Gentle aeration or increased flow rate should be used to keep dissolved oxygen concentration above 4 ppm unless there are reliable data to indicate that lower dissolved oxygen levels would occur for a substantial period of time in the field during the proposed disposal operation or that lower levels occur naturally at the site.

21. After 10 days, turn off the flow of water and siphon the

sediments through a 0.5-mm screen. Mix the material retained on the screen with some seawater and search it thoroughly for animals. Consider animals alive if they show any response to gentle probing of a sensitive part. Sublethal effects such as partial paralysis should also be recorded. For many benthic species, an appropriate sublethal response criterion is the inability to burrow in sediments or to excavate burrows. Specimens not recovered must be considered dead. All crustaceans molt at regular intervals, shedding a complete exoskeleton. Care should be taken not to count an exoskeleton as a dead animal. Dead animals may decompose or be eaten between observations. Therefore, always count living, not dead animals. A sample of recovered specimens not needed for further analysis should be preserved in formalin if needed for verification of species identification. If animals from the bioassay are to be used in estimating bioaccumulation potential, the survivors should be gently and rapidly counted and treated as discussed in Appendix G, beginning with paragraph 12.

Analysis and Interpretation of Results

22. According to Section 227.13 of the Register, dredged material can be considered environmentally acceptable for ocean disposal only if bioassay and mixing results indicate that the limiting permissible concentration (LPC) will not be exceeded (Section 227.27). The primary objective of the bioassay is to determine if there is a statistically significant decrease in mean survival of all species in the dredged material treatment(s) relative to the control.

23. It is important to realize that a statistically significant effect in a bioassay does not necessarily imply that an ecologically important impact would occur in the field. This must be kept in mind when interpreting results, particularly in cases where a difference of small magnitude between survival in the control and test sediments is shown to be statistically significant. At present there is no quantitative method for estimating the magnitude of such a difference that might reliably be assumed to predict the occurrence of adverse impact

on animals in the field. However, there is a general feeling among many scientists that differences between control and treatment survival of 10 percent are necessary in most cases before predictions of probable impact can be made. Of course, regardless of the magnitude of the difference between mean survival levels, if the means are not shown to be statistically different, they must be regarded as equal.

24. The statistical example given later analyzes total mortality of all species. The sensitivity of this procedure may be increased, if desired, by blocking the data on species using the method given in Table 11.7 on page 327 of Sokal and Rohlf.[3] Survival of individual species can be analyzed by the same statistical tests as the combined survival of all five test species. The relative sensitivity of the different species could reflect phylogenetic susceptibility to certain toxicants. If differences in mean survival are not significant, analysis of sublethal responses such as paralysis, inability to burrow, or bioaccumulation may indicate potentially unacceptable impact. Such responses may also be analyzed by the statistical method presented below.

Data presentation

25. Present data in a table giving the scientific name of the test species, the number of animals seeded, and the percent of animals recovered alive from each aquarium. If greater than an average of 10-percent mortality occurs in the controls, all data must be discarded and the experiment repeated. The 10-day test period represents a major portion of the life span of some species such as mysid shrimp, and unless the test is begun with juveniles, mortality greater than 10-percent may be expected from natural causes. Unacceptably high control mortality indicates the presence of important stresses on the organisms other than the material being tested, such as injury or disease, stressful physical or chemical conditions in the test containers, improper handling or acclimation, or perhaps an adverse impact from an unsuitable or contaminated reference sediment.

26. If less than 10-percent mortality occurs in the controls, the data may be evaluated. It is possible that the solid phase of some dredged material will produce no mortality, and total survival in the

dredged material may be equal to or higher than survival in the reference substrate controls. If so, visual inspection of the data is adequate, and no statistical analyses are needed. Such cases have been documented and in no way reflect on the quality of the bioassay, simply indicating an absence of lethal effects of the dredged material.

Statistical analysis

27. If survival in the reference substrate control is higher than that in the dredged material, the data must be compared statistically. The following example is a hypothetical case in which dredged material from three sampling stations in the dredging site were analyzed, giving a total of four treatments including the reference substrate. The hypothetical data are shown in Table F2.

Table F2

Hypothetical Results of a Solid Phase Dredge Material Bioassay Using Three Samples of Dredged Material

	Total Surviving Animals			
Replicate (n = 5)	Reference Substrate Control	Dredged Material 1	Dredged Material 2	Dredged Material Sample 3
1	85	71	94	79
2	90	67	88	72
3	92	69	94	83
4	96	61	90	67
5	92	84	93	77
sum of data = ΣX =	455	352	459	378
mean, $\bar{X} = \frac{\Sigma X}{n}$ =	91.0	70.4	91.8	75.6
sum of squared data = ΣX^2 =	41,469	25,068	42,165	28,732
corrected sum of squares, $CSS = \Sigma X^2 - \frac{(\Sigma X)^2}{n}$ =	64.0	287.2	28.8	155.2
variance, $S^2 = \frac{CSS}{n-1}$ =	16.0	71.8	7.2	38.8

28. An analysis of variance (ANOVA) is used to compare the mean survival in the reference substrate control to the mean survival in the dredged material samples. In cases where it is felt that adequate

information may be obtained from one sampling station in the dredging site, the data may be analyzed by a t-test analogous to that discussed in paragraphs 20 through 27 of Appendix D.

29. Before an ANOVA can be performed, it is necessary to determine whether the variances of the data sets are homogeneous. This is determined by Cochran's test for the homogeneity of variances. The C-value is calculated as the ratio of the largest variance to the sum of all variances.

$$C = \frac{S^2_{max}}{\Sigma S^2} = \frac{71.8}{133.8} = 0.5366$$

where

S^2_{max} = largest variance among the data sets

ΣS^2 = sum of all the variances

30. This C-value is evaluated by comparing it to the tabulated C-value given in the table that is Enclosure 1 to Appendix D. In the table, k is the number of treatment variances summed in the denominator (4 in this case), and ν is one less than the number of observations contributing to each variance (5 - 1 = 4 in this case). Therefore, the tabulated C-value in this example is 0.6287.

31. Since the calculated C-value is smaller than the tabulated C-value, the calculated value is not significant at the 95-percent confidence level, and the variances may be considered homogeneous. If the calculated C-value is larger than the tabulated C-value, the variances are not homogeneous. In this case before any ANOVA calculations are performed, a transformation should be performed on all data in order to achieve homogeneity of variances. This may be done by obtaining either the natural logarithm of (X + 1), or the arcsin $\sqrt{X}$, where X is the datum. If the arcsin $\sqrt{X}$ transformation is to be used, the data must first be converted to percents and expressed as decimal fractions (i.e., 0.92 survival, not 92-percent survival). Recalculate the C-value using data transformed by either of these methods. If variances are now found to be homogeneous, use the transformed data in all ANOVA calculations. If

variances are still nonhomogeneous, an approximate test of the equality of means given by Sokal and Rohlf in their Box 13.2[3] should be used.

32. ANOVA equations and calculations for the data of Table F2 are given in Table F3. The values on the third line of the table (Total) should be the same whether they are calculated by the equation or obtained by summing the corresponding treatment and error values, thus providing an easy means of checking the accuracy of the calculations. The calculated F-value is evaluated by comparison with the tabulated F-value from Reference 4 at the 0.05-probability level with the appropriate df. The df's are those given for the treatments and error, respectively, in Table F3. The tabulated F-value with 3 and 16 df is shown at the bottom of Table F3. Since the calculated F-value exceeds the tabulated value, there is a statistical difference between mean survival among the four sets of data. If the calculated F-value had been equal to or less than the tabulated value, there would be no statistical differences between survival in the reference substrate controls and any of the dredged material samples. In that case, the analysis would be complete at this point with no indication of potential adverse impact of the solid phase.

33. When the calculated F-value exceeds the tabulated value, it is then necessary to determine which dredged material means differ significantly from the reference substrate control mean. This may be done by the Student-Newman-Keuls multiple-range test given by Sokal and Rohlf in their Box 9.9.[3] Least significant ranges (LSR) used in this process are the product of the pooled standard error of the group mean ($S_{\bar{X}}$) and the studentized ranges (Q) given in Rohlf and Sokal's Table U.[4]

$$S_{\bar{X}} = \sqrt{\frac{\text{MS error}}{n}} = \sqrt{\frac{33.45}{5}} = 2.59$$

where the terms are taken from Table F3.

34. At the 0.05-level of significance, the Q and LSR values for the number of means (K) = 2, 3, and 4 are:

Table F3

ANOVA Equations and Calculations for the Data from Table F2

Source of Variation	Degree of Freedom		Sum of Squares (SS)		Mean Square (MS)		F	
	Equation	Value	Equation	Value	Equation	Value	Equation	Value
Treatments†	(a-1)	3	$\Sigma \frac{(\Sigma X)^2}{n} - \frac{(\Sigma\Sigma X)^2}{\Sigma n}$	1762.0	$\frac{SS_{treatment}}{a-1}$	587.33	$\frac{MS_{treatment}}{MS_{error}}$	17.56*
Error	a(n-1)	16	ΣCSS	535.2	$\frac{SS_{error}}{a(n-1)}$	33.45		
Total	(an)-1	19	$\Sigma(\Sigma X^2) - \frac{(\Sigma\Sigma X)^2}{\Sigma n}$	2297.2				

†Number of treatments a = 4

*$F_{.05(3,\ 16)} = 3.24$

	K		
	2	3	4
Q(Rohlf and Sokal, Table U[4]) =	2.998	3.649	4.046
$S_{\bar{X}}$ =	2.59	2.59	2.59
LSR = $QS_{\bar{X}}$ =	7.76	9.45	10.48

35. The multiple-range test is completed by arranging for four treatment means in increasing order and then comparing the difference between means with the LSR for the number of means (K) in the range separating the two being compared. That is, for two adjacent means K = 2; for comparing means separated by one mean, there is the intervening mean and the two being compared, so that K = 3. It is necessary to compare each dredged material mean to the control mean, but not to compare treatment means among themselves. Such comparisons between treatment means are not necessary for permitting decisions, but could provide useful managerial information by distinguishing sediments causing a great impact from those causing a smaller but still statistically significant impact. The comparison of treatment means to the control for the above example is given below.

Treatment Means Computed from Table F2

$\bar{X}_1$	$\bar{X}_3$	$\bar{X}_{control}$	$\bar{X}_2$
70.4	75.6	91.0	91.8

Mean Comparison		
K	LSR	Difference Between Means
3	9.45	$\bar{X}_c - \bar{X}_1 = 91.0 - 70.4 = 20.6$*
2	7.76	$\bar{X}_2 - \bar{X}_c = 91.8 - 71.0 = 0.8$ n.s.
2	7.76	$\bar{X}_c - \bar{X}_3 = 91.0 - 75.6 = 15.4$*

Note: Entry of * indicates difference between means is significant at the 0.05-probability level; n.s. indicates difference is not significant

36. When the difference between two means is greater than the LSR, the difference between those means is statistically significant at the 0.05-probability level. Therefore, the multiple-range test has shown

that the mean survival in dredged material samples 1 and 3 is statistically lower than survival in the reference substrate control, while the survival in dredged material sample 2 is not statistically different from that in the control. The difference between survival in the control and samples 1 and 3 is greater than the minimum difference generally considered to indicate the probability of biologically important effects, as discussed in paragraph 23. Had the treatment and control means shown a statistically significant difference of only a few percent, the prediction of important effects would be much more tenuous.

Limiting permissible concentration

37. The LPC of the solid phase of dredged material is defined in paragraph 227.27(b) of the Register as that concentration of solids that will not cause "unreasonable effects" beyond the disposal site boundary. Paragraphs 227.29(a) and (b)(2) clearly imply that the initial dispersion of the solid phase that occurs within 4 hr after disposal is to be considered in determining whether the LPC would be exceeded. At present there are no objective methods for considering initial mixing and dispersion in the interpretation of solid phase bioassay data. Therefore, this guidance takes the environmentally protective approach that the LPC of the solid phase is operationally determined by the results of the solid phase bioassays. If the difference in mean survival between animals in the control and test sediments is statistically significant and greater than 10 percent, as in this example, the LPC would be considered to be exceeded, and the bioassay would be considered to have shown the material to have a real potential for causing environmentally unacceptable impacts on benthic organisms. This method of interpretation based on statistically significant mean differences of at least 10 percent should be used only with the solid phase bioassay technique. The level of 10 percent is subject to revision if warranted by further studies and experience, but is the level presently considered most realistic for environmental protection purposes by those most familiar with solid phase dredged material bioassays.

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APPENDIX G: GUIDANCE FOR ASSESSING BIOACCUMULATION POTENTIAL

Introduction

1. The ocean disposal criteria require that the potential for bioaccumulation of contaminants from dredged material be evaluated in the technical assessment of permit applications. This requires predicting whether there will be a cause-and-effect relationship between an animal's presence in the area influenced by the dredged material and a significant elevation of its tissue content or body burden of contaminants above that in similar animals not influenced by the disposal of dredged material. That is, it must be predicted whether an animal's exposure to the influence of the dredged material is likely to cause a meaningful elevation of contaminants in its body.

2. A variety of laboratory research methods for measuring bioaccumulation are presently undergoing modification and evaluation as regulatory tools. All such methods require one month or more for completion and provide no quantitative method for considering field conditions such as mixing in the interpretation of the results, as required by the Register. Field sampling programs overcome the latter difficulty since the animals are exposed to the conditions of mixing and sediment transport actually occurring at the disposal site in question. The former difficulty is also overcome if organisms already living at the disposal site are utilized in the bioaccumulation studies. The use of this approach for predictive purposes is technically valid only where there exists a true historical precedent for the proposed operation being evaluated. That is, it can be used only in the case of maintenance dredging where the quality of the sediment to be dredged is considered not to have deteriorated or become more contaminated since the last dredging and disposal operation. In addition, the disposal must be proposed for the site at which the dredged material in question has been previously disposed or for a site of similar sediment type supporting a similar biological community.

3. Considering these limiting conditions and following the

procedure given below, it is possible to assess bioaccumulation by animals that have spent major portions of their life in or on a sediment very similar to the sediment in question under the physical and chemical conditions actually occurring at the disposal site. Caged animals of suitable species may also be placed at appropriate stations in and around the disposal site, but this will require a substantial exposure time before analysis. If the conditions discussed above cannot be met in the field, a general approximation of bioaccumulation potential may be obtained as described later in this appendix from animals used in the suspended particulate or solid phase bioassays.

Field Assessment of Bioaccumulation Potential

Apparatus

4. The following is a general description of the major items required. Additional miscellaneous equipment will have to be furnished.

   a. A vessel capable of operating at the disposal site and equipped to handle benthic sampling devices. Navigation equipment must be sufficient to allow precise positioning.

   b. Sampling devices such as a Smith-MacIntyre or other benthic grab. Corers are less satisfactory since they sample a smaller surface area and have a greater penetration than is needed.

   c. Stainless steel screens of 1-mm mesh to remove animals from the sediment.

   d. Tanks sufficient for transporting the animals to the laboratory in collection site water.

   e. Laboratory facilities for holding the animals prior to analysis.

   f. Chemical and analytical facilities as required for the desired analyses.

Species selection

5. The species selected for analysis must occur in sufficient numbers for collection of an adequate sample at all stations. The same species must be collected at all stations since comparisons of bioaccumulation cannot be made across species lines.

6. For each species at each station, a minimum of several grams

of tissue, as indicated in the references given in paragraph 20, must be collected to provide sufficient sample to allow measurement of chemical concentrations. In samples that do not contain sufficient tissue, it will be impossible to quantify the amount of contaminant present. Since data in the form of "concentration below detection limits" is not quantitative, it is vital that sufficient tissue to allow definitive measurement of concentration be collected for each species at each station. It is also important that exactly equal masses of tissue be analyzed for each station. If possible, several samples of sufficient size for analysis should be collected at each sampling station in order to provide a statistical estimate of variability in tissue content of the contaminants of concern. The collection of more than one sample per station, however, may prove impossible in practice if small organisms must be used or if suitable organisms are not abundant at the disposal site. In such cases the use of caged animals, as discussed in paragraph 10, may be advisable.

7. It is desirable to select the largest appropriate species so as to minimize the numbers and collection effort required. However, highly mobile epifauna (such as crabs, lobsters, shrimp, and fish) should not be used, since their location when collected cannot be related to their body burden at the time of collection in any potential cause-and-effect manner. Therefore, relatively immobile species that are fairly large, such as bivalves, some gastropods, large polychaetes, etc., are the most desirable organisms. Any relatively immobile species collectable in sufficient numbers at all stations may be used, but the required collection effort increases sharply as organism size decreases.

Sampling design and conduct

8. Sufficient tissue to obtain definitive body burden values must be collected from each of at least three stations within the disposal site boundaries and from each of at least six stations outside the disposal site. The stations outside the site must be located in areas with a substrate sedimentologically similar to that within the disposal site. These stations outside the disposal site will serve two purposes. If the direction of net bottom transport at the site is known, at least

three stations should be located in a substrate similar to that within the site and in the path of transport away from the site. The data from these stations will provide an indication of uptake of any contaminants transported out of the disposal site. At least three stations must also be located in an uncontaminated sediment sedimentologically similar to that within the site, but in a direction opposite that of the net bottom transport. Data from these sites will provide a reference level of contaminants in tissues to which those levels found in and downstream from the disposal site may be compared. If the direction of net bottom transport is not known, at least six stations surrounding the disposal site should be established in sediments sedimentologically similar to those within the disposal site.

9. In all cases it is mandatory that several stations be sampled, rather than collecting all of the animals at one station. This will provide a measure of the variability that exists in tissue concentrations in the animals in the area. Samples from all stations should be collected the same day if possible and in any case within four days.

10. If caged animals placed around the disposal site are utilized instead of free animals living there naturally, all the considerations of paragraphs 8 and 9 must be evaluated in selecting the sampling stations, including the sedimentological similarity of the substrate at all stations. The cages must be designed and positioned such that the animals are able to burrow or establish their natural relationship to the sediment in order to truly evaluate the influence of the dredged material on bioaccumulation potential. Cages should not be constructed of metal or coated with material that may leach the contaminants of concern. They must be anchored and marked on the surface so that they can be reliably located and recovered.

11. When the collection vessel has been positioned, repeated collections are made at the same spot until an adequate sample is obtained. The sediment obtained by the sampler is hosed through 1-mm stainless steel screens, and the retained individuals of the desired species are placed in holding tanks. In all cases no animal with any indication of injury should be retained.

12. Return the animals to the laboratory, being careful to label the samples clearly and keep them separated and to maintain nonstressful temperature and dissolved oxygen levels. In the laboratory, maintain the samples in clean water in separate containers. No sediment is placed in the containers and the animals are not fed. Any organisms that die must be immediately discarded. Fecal material is siphoned from the aquaria twice daily until little more is produced, indicating that all material has been voided from the digestive tracts. This probably will be completed within 2 to 3 days after collection, and sooner with small animals. A more desirable procedure, if animals are large enough to make it practical, is to excise the digestive tracts soon after collection rather than allowing the animals to excrete their contents. It is necessary to empty or remove the digestive tracts since material therein may well contain inert constituents and the contaminants of concern in forms that do not become biologically available during passage through the digestive tract. Such material would also probably be unavailable while passing through the digestive tract of any predator that might have ingested the animals being analyzed. Therefore, since the digestive tract content has not been incorporated into the tissue, it would give an artificially high indication of bioaccumulation if it were included in the analysis.

13. The shells or exoskeletons of molluscs or crustaceans are removed and not included in the analysis. These structures generally contain low levels of contaminants and would contribute weight but little contaminants if they were included in the analysis. This would give an artificially low indication of bioaccumulation.

Analysis and interpretation

14. Preparation and analysis of tissues are by the procedures given in the "Chemical Analysis" section of this appendix. The section on "Data Analysis and Interpretation" gives guidance on these matters.

Laboratory Assessment of Bioaccumulation Potential

Sampling design and conduct

15. This approach should be taken only in those cases where a true historical precedent for the proposed operation does not exist (as discussed in paragraph 2). The considerations of paragraphs 5, 6, and 7 should be kept in mind when selecting bioassay species to be used for laboratory assessments of bioaccumulation potential.

16. Animals from solid or suspended particulate phase bioassays may be used, but it is considered unlikely that important bioaccumulation would occur at the disposal site from the latter phase, since animals would be exposed to it for such short periods due to dilution. At the end of the bioassay, surviving animals from the replicate controls are treated in a manner corresponding to the separate reference samples in the field assessment outlined earlier. Survivors from the replicate sediment-exposure aquaria correspond to the samples from the disposal site. In the case of suspended particulate bioassays, survivors from the first replicate of all test medium concentrations are pooled to make one sample corresponding to a disposal site sample; survivors from the second replicate of all test medium concentrations are pooled to make the second disposal site sample, etc.

17. At the end of the bioassay, each sample is placed in separate aquaria in clean, sediment-free water to void the digestive tracts, as discussed in paragraph 12. Each replicate from the bioassay is treated as if it was a sample from the field assessment discussed earlier. If very small animals are to be analyzed, more than the minimum number specified for the bioassay may have to be used, or more replicate aquaria may be established in the bioassay. The considerations of paragraph 13 also apply to bioassay organisms used in assessing bioaccumulation potential.

Analysis and interpretation

18. Preparation and analysis of tissues are by the procedures given in the "Chemical Analysis" section of this appendix. The section on "Data Analysis and Interpretation" gives guidance on these matters.

Chemical Analysis

Constituents to be assessed

19. The chemical constituents to be assessed for bioaccumulation are those constituents deemed critical by the District Engineer and Regional Administrator after considering known inputs to the sediment to be dredged. The following constituents, discussed in Section 227.6 of the Register, are of particular concern and should be assessed for bioaccumulation whenever the District Engineer and Regional Administrator have any reason to believe they may be of concern in the sediment in question.

a. Organohalogen compounds (PCB's, DDT, etc.)

b. Mercury and its compounds

c. Cadmium and its compounds

d. Petroleum hydrocarbons

e. Known or suspected carcinogens, mutagens, or teratogens. (This is a very poorly defined group of materials for which specific analytical procedures are not generally available.)

Procedures

20. Referenced standard procedures for specific constituents are given in Table G1. These references should be consulted for detailed guidance on amount of tissue required for analysis of each constituent of concern, methods of sample preparation and analysis, and data presentation.

Data Analysis and Interpretation

21. Complete tissue concentration data for all samples should be presented as in Table G2. A separate analysis must be conducted for each chemical constituent and each animal species. This example utilizes laboratory bioaccumulation data from analyzing the survivors of the hypothetical solid phase bioassay presented in Appendix F. The control and the dredged material samples from three sites were each replicated five times, corresponding to the five replicates used here.

Table G1

Procedural References for Analytical Methods for Tissue Analyses of Organic Materials

Material	Reference 1	Reference 2	Other References
BHC	Sections 211, 212	Section 5A	
Heptachlor	" " "	"	3, 4
DDD, DDE, DDT	" " "	"	5, 6, 7
Chlordane	" " "	"	8
Dieldrin	" " "	"	7
Endrin	" " "	"	9
Toxaphene	" " "	"	4
PCB	Sections 211, 212, 251	"	10
Mirex	Sections 211, 212	"	11
Methoxychlor	" " "	"	
Mercury and its compounds			12
Cadmium and its compounds			12
Petroleum hydrocarbons:			
Aliphatic			13
Aromatic			13

Table G2

Hypothetical Results* of a Laboratory Assessment of Bioaccumulation Potential

Replicate (n = 5)	Tissue Concentration, ppm (wet wt) Control	Dredged Material Sample 1	2	3
1	0.15	0.27	0.25	0.15
2	0.08	0.42	0.38	0.12
3	0.38	0.24	0.52	0.24
4	<0.05	0.37	0.47	0.14
5	0.23	0.49	0.61	0.30
sum of data, ΣX =	0.89	1.79	2.23	0.95
mean, $\bar{X} = \frac{\Sigma X}{n}$ =	0.18	0.36	0.45	0.19
sum of squared data = ΣX^2 =	0.2287	0.6839	1.0703	0.2041
corrected sum of squares, $CSS = \Sigma X^2 - \frac{(\Sigma X)^2}{n}$ =	0.0703	0.0431	0.0757	0.0236
variance, $S^2 = \frac{CSS}{n-1}$ =	0.0176	0.0108	0.0189	0.0059

* The constituent measured and the animal species used in the assessment must be identified.

Small organisms were used; in one case, tissue concentration of the constituent of concern was below detection limits. Such data are nonquantitative and cannot be used in statistical analyses. However, the arbitrary but environmentally protective assumption made in such cases is that the actual concentration in the sample was only slightly less than the detection limit, and the detection limit is used as if it was the datum.

22. To determine whether there is an indication of bioaccumulation potential, it is necessary to make statistical comparisons of the tissue concentrations in the controls to those in animals exposed to the dredged material. It is possible that in some cases the mean tissue concentration in one or more of the dredged material samples may be less than or equal to that in the controls. Such cases have been documented and in no way reflect adversely on the quality of the evaluation, but simply

give no indication of bioaccumulation potential for the constituent, species, and sediment sample in question.

23. If tissue concentration in any of the dredged material samples is higher than that in the controls, the data must be compared statistically. An analysis of variance (ANOVA) is used to compare the mean tissue concentration in animals from the reference substrate control to the mean tissue concentration in animals exposed to each dredged material sample. Before an ANOVA can be performed, it is necessary to use Cochran's test to determine whether the variances of the data sets are homogeneous. This is determined by calculating the C-value, defined as the ratio of the largest variance to the sum of all the variances. In this case:

$$C = \frac{S^2_{max}}{\Sigma S^2} = \frac{0.0189}{0.0532} = 0.3553 \qquad \text{(G1)}$$

where

S^2_{max} = largest variance among the data sets

ΣS^2 = sum of all the variances

The calculated C-value is evaluated by comparing it to the C-value given in the table in Enclosure 1 to Appendix D. In the table, k is the number of treatment means summed in the denominator (4 in this case) and ν is one less than the number of observations contributing to each variance (5 - 1 = 4 in this case). Therefore, the tabulated value for C in this example is 0.6287.

24. Since the calculated C-value is smaller than the tabulated C-value, the calculated value is not significant at the 95-percent confidence level, and the variances may be considered homogeneous. If the calculated C-value is larger than the tabulated C-value, the variances are not homogeneous. In such cases, before any ANOVA calculations are performed, a transformation should be performed on all data in order to achieve homogeneity of variances. The transformation is performed on each datum by obtaining the natural logarithm of (X + 1), where X is the datum. Recalculate the C-value using the transformed data. If

variances are now found to be homogeneous, use the transformed data in all ANOVA calculations. If the variances are still nonhomogeneous, an approximate test of the equality of means given by Sokal and Rohlf in their Box 13.2[14] should be used.

25. ANOVA equations and calculations for the data of Table G2 are given in Table G3. The values on the third line of the table (Total) should be the same whether they are calculated by the equation or obtained by summing the corresponding treatment and error values, thus providing an easy means of checking the accuracy of the calculations. The calculated F-value is evaluated by comparison with the tabulated F-value[15] at the 0.05-probability level with the appropriate degrees of freedom (df). The df's are those given for the treatments and error, respectively, in Table G3. The tabulated F-value with 3 and 16 df's is shown at the bottom of Table G3. Since the calculated F-value exceeds the tabulated value, there is a statistical difference between mean tissue concentrations among the four sets of data. If the calculated F-value had been equal to or less than the tabulated value, there would be no statistical differences between tissue concentration in the reference substrate controls and any of the dredged material samples. In that case, the analysis would be complete at this point with no indication of potential bioaccumulation from the dredged material in question.

26. When the calculated F-value exceeds the tabulated value, it is then necessary to determine which dredged material means differ significantly from the reference substrate control mean. This may be done by the Student-Newman-Keuls multiple-range test given by Sokal and Rohlf in their Box 9.9.[14] Least significant ranges (LSR) used in this process are the product of the pooled standard error of the group mean $S_{\bar{X}}$ and the studentized ranges Q given in Rohlf and Sokal's Table U.[15]

$$S_{\bar{X}} = \sqrt{\frac{\text{MS error}}{n}} = \sqrt{\frac{0.0133}{5}} = 0.0516 \qquad \text{(G2)}$$

where the terms are taken from Table G3.

27. At the 0.05 level of significance, the Q and LSR values for K = 2, 3, and 4 items are:

Table G3

ANOVA Equation and Calculations for the Data of Table G2

Source of Variation	df		Sum of Squares SS		Mean Square MS		F	
	Equation	Value	Equation	Value	Equation	Value	Equation	Value
Treatments†	(a-1)	3	$\Sigma \frac{(\Sigma X)^2}{n} - \frac{(\Sigma\Sigma X)^2}{\Sigma n}$	0.2573	$\frac{SS_{treatment}}{a-1}$	0.0858	$\frac{MS_{treatment}}{MS_{error}}$	6.45*
Error	a(n-1)	16	ΣCSS	0.2127	$\frac{SS_{error}}{a(n-1)}$	0.0133		
Total	(an)-1	19	$\Sigma(\Sigma X^2) - \frac{(\Sigma\Sigma X)^2}{\Sigma n}$	0.4700				

† Number of treatments a = 4

* $F_{.05(3,16)} = 3.24$

	K		
	2	3	4
Q(Rohlf and Sokal, Table U[15]) =	2.998	3.649	4.046
$S_{\bar{X}}$ (equation G2) =	0.0516	0.0516	0.0516
LSR = $QS_{\bar{X}}$ =	0.1547	0.1883	0.2088

28. The multiple-range test is completed by arranging the four treatment means in increasing order and then comparing the difference between means with the LSR for the number of means K in the range separating the two being compared. That is, for two adjacent means K = 2 and for comparing means separated by one mean, there is the intervening mean and the two being compared, so that K = 3. It is necessary to compare each dredged material mean to the control mean but not to compare treatment means among themselves. Such comparisons between treatment means are not necessary for permitting decisions, but could provide useful managerial information by distinguishing sediments with high bioaccumulation potential from those with a lesser but still statistically significant bioaccumulation potential. The comparison of treatment means to the control for the above example is given in the following tabulation.

Treatment Means from Table G2			
$\bar{X}_{control}$	$\bar{X}_3$	$\bar{X}_1$	$\bar{X}_2$
0.18	0.19	0.36	0.45

Mean Comparison		
K	LSR	Difference Between Means
2	0.1547	$\bar{X}_3 - \bar{X}_c = 0.19 - 0.18 = 0.01$ n.s.
3	0.1883	$\bar{X}_1 - \bar{X}_c = 0.36 - 0.18 = 0.18$ n.s.
4	0.2088	$\bar{X}_2 - \bar{X}_c = 0.45 - 0.18 = 0.27$*

Note: Entry of n.s. indicates difference is not significant at the 0.05-probability level;
* indicates difference is significant

29. When the difference between two means is greater than the LSR, the difference between those means is statistically significant at the 0.05-probability level. Therefore, the multiple-range test has shown

that the mean tissue concentration of the constituent of concern in animals exposed to dredged material sample 2 is statistically higher than the corresponding concentration in animals exposed to the control sediment. Tissue concentrations of this constituent in animals exposed to dredged material samples 1 and 3 were not statistically higher than in the control animals.

30. The ANOVA calculations and mean comparison given above may be used for data analysis in all cases involving two or more treatments, provided that the same number of samples occurs in each treatment. The ANOVA calculations for studies in which the same number of samples does not occur in each treatment are given by Sokal and Rohlf in their Box 9.1.[14] Unequal numbers of replicate samples may occur in field evaluations where the direction of net bottom transport is not known and samples outside the disposal site are located in all directions from the site. In such cases, those stations outside the site having the highest tissue concentrations cannot arbitrarily be assumed to lie in the direction of net bottom transport, unless this is also indicated by independent evidence. Otherwise the only analysis possible is to compare the mean tissue concentration at the stations within the disposal site to the pooled mean tissue concentration at all stations outside the site. That is, two samples containing different numbers of observations will be compared. When the direction of net bottom transport is known, three mean tissue concentrations will be compared. These will be from samples within the site, samples outside the site in the direction of net bottom transport, and samples outside the site and not influenced by net transport from the site.

31. In the example given in paragraphs 21 through 29, by comparison to the control animals, animals in one of the dredged material samples had elevated tissue concentrations of the constituent of concern, and those in the other two samples did not. Therefore, there is a potential for bioaccumulation of this chemical by this species of animal from sediments at one site in the dredging area.

32. At present there are very little data for marine species upon which to base an evaluation of the meaning of a specific concentration

of a particular contaminant in the species in question. The only such levels that are fixed from a regulatory viewpoint are those levels set by the Food and Drug Administration for fish and shellfish for human consumption. Therefore, this guidance recommends the environmentally protective approach of assuming that any statistically significant differences in tissue concentrations between control and exposed organisms are a potential cause for concern. It should be kept in mind, however, that at present tissue concentration of most constituents in most species cannot be quantitatively related to biological effects. Therefore, in making the final assessment of bioaccumulation, the District Engineer and the Regional Administrator must objectively consider the magnitude of bioaccumulation shown, the toxicological significance of the material(s) bioaccumulated (i.e., arsenic would be of greater concern than iron), the proportion of sediment sampling sites which produce uptake, the number of different constituents bioaccumulated from the sediment in question, the position in human and nonhuman food webs of the species showing uptake, the presence of motile species at the site that might serve as transportation vectors removing bioaccumulated materials from the disposal area, and other factors relevant to the particular operation in question.

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APPENDIX H: ESTIMATION OF INITIAL MIXING

Introduction

1. The Register recognizes the fact that the oceanic environment is physically dynamic and that materials dumped into it will be dispersed, mixed, and diluted to some degree. Therefore, all evaluative procedures must be interpreted in light of the initial mixing expected at the disposal site. Initial mixing is defined (paragraph 227.29(a) of the Register) to be that dispersion or diffusion of liquid, suspended particulate, and solid phases of a material that occurs within 4 hr after disposal. The limiting permissible concentration (Section 227.27) shall not be exceeded beyond the boundaries of the disposal site during the 4-hr initial mixing period and shall not be exceeded at any point in the marine environment after initial mixing. A series of methods, discussed in the order of preference shown in Section 227.29 of the Register, may be used to estimate the maximum concentration of the liquid and suspended particulate phases found at the disposal site after initial mixing. Since no objective method has been devised for incorporating initial mixing of the solid phase into the interpretation of bioassay results, no calculations are presented here for estimation of the initial mixing of the solid phase.

Initial Mixing Calculations

Mathematical models using specific field data (227.29(a)(1))

2. The first and most preferred method requires the use of comprehensive field data relevant to the proposed disposal operation in conjunction with an appropriate mathematical model for adequate prediction of initial mixing and dispersion. However, the amount of field data necessary for adequate prediction of dispersion and diffusion is substantial, and such predictions require a detailed understanding of tides, currents, waves, water column stratification, and climatic conditions at the disposal site.

3. Description of the WES mathematical models. The Dredged Material Research Program (DMRP) of the U. S. Army Engineer Waterways Experiment Station (WES) has modified two numerical models, now being field verified, to predict the short-term fate of dredged material discharged in the marine environment. One model simulates an instantaneous discharge from a rapidly emptying barge or hopper. The other simulates a continuous fixed or moving jet discharge from a slowly emptying vessel. The models can be applied to disposal sites in enclosed bodies of water, sites with depth variations, sites whose flow regimes vary in three dimensions and in time, and those disposal sites where ambient density varies in time.

4. It should be noted that neither adequate calibration nor verification of the models has been completed at this time. However, from limited demonstrations and calibrations, the models have been shown conceptually to be capable of predicting the dynamic physical processes associated with various dredged material disposal operations in the marine environment. The models will not be generally available until the calibrations and verifications necessary to ensure the accuracy of model predictions have been concluded. This is expected to be completed by early 1978. The availability of the models for general use will be announced. At that time the WES models will become the preferred means of estimating initial mixing for most disposal operations whose size or potential impact warrant this level of sophistication. The release zone method described in paragraphs 10 through 28 may still be acceptable for small projects of little anticipated impact.

5. In consideration of those who are planning a field data-collection program in anticipation of use of the WES mathematical models or who may think adequate data are available for input to the models discussed in paragraph 3, a brief description of the two models is given with specific requirements of the necessary input data for optimal model utilization. Both models characterize the behavior of released dredged material with a convective descent phase in which the cloud has a high density relative to the disposal site water and is dominated by gravitational forces; a dynamic collapse phase in which horizontal spreading

dominates, usually initiated when the descending cloud impacts the bottom; and a long-term dispersion phase, which is dominated by the ambient currents and turbulent diffusion at the disposal site, rather than the forces of the disposal operation.

6. WES model input requirements. Ocean disposal of dredged material is usually made by barges or scows and hopper dredges. When a barge releases its material in a stationary mode (i.e., not underway), the disposal can be assumed to be an instantaneous dump for modeling purposes and the instantaneous model can be used to simulate this operation with the proper input parameters. Moving barges having to open several doors to release all the material would be best described by the moving jet model. The moving jet model also best describes most hopper dredge disposal operations in which one or two doors are usually opened at a time and up to thirty minutes may be required to remove all the material. The characteristics of each specific disposal operation will determine the model appropriate to its simulation and thus will determine the model input data required. Adequate input data can be obtained only with a comprehensive understanding of the factors affecting the dredging and disposal operations and by a thorough field sampling program based on this understanding.

7. Input data required to run the models can be categorized as (a) data describing the actual disposal operation, (b) characterization of the dredged material, (c) a description of the ambient environment, and (d) model coefficients.

a. Disposal data. For the instantaneous or stationary discharge model, the grid position of the barge on the horizontal grid, the radius of the initial cloud, the depth below the surface where the material is released, and the initial velocity of the cloud are required. Generally the radius of the initial cloud will be determined by the total volume of the barge. The fixed or moving jet discharge model requires the initial position of the discharge, the vessel course and speed, the orientation and depth of the discharge point in the water column, the radius of the initial jet, the flow rate, and the total time required for complete discharge.

b. Characterization of dredged material. The models will accept up to twelve solid fractions (grain sizes), a fluid

component, and a conservative chemical constituent (e.g., ammonia) if desired. The concentration, density, fall velocity, void ratio, and an indicator of cohesion must be input for each solid fraction. If a conservative chemical constituent is to be used, its initial concentration in the liquid phase and a background concentration in the disposal site water must be given. In addition, the bulk density and aggregate void ratio of the dredged material must be determined. It is not necessary to input all twelve solid fractions to adequately simulate the dispersion processes during disposal, especially when there is a cohesive fraction included. An important but difficult value to obtain prior to dredging is the bulk density of the total volume of dredged material in the barge. If large volumes of water are dredged with the actual material, the bulk density values will be substantially reduced.

c. Description of ambient environment. An ambient density profile must be supplied and, at each horizontal grid point, water depth and a current velocity profile are required. The level of sophistication here is optional, as there are three different forms of velocity input, the depths may be constant or variable in space, and the density profile may vary with time or remain constant.

d. Model coefficients. The models contain recommended average values for fourteen coefficients, which the user should change only if justified by case-specific data.

Similar field data and modeling (227.29(a)(2))

8. The second method of initial mixing estimation permitted by the Register allows field data determined for a material of similar characteristics to be used in conjunction with an appropriate model. There may be certain similarities between dredged material disposal operations in different regions of the country that may allow the use of similar input data to simulate a proposed disposal operation; however, the similarities have not yet been documented that could justify this method for prediction of dredged material dispersion. Certainly, there is no justification for using input data developed for other waste material in attempts to predict dredged material dispersion.

Theoretical relationships (227.29(a)(3))

9. When no field data are available, the Register permits consideration of theoretical oceanic turbulent diffusion relationships in

order to estimate initial mixing. The state-of-the-art of dredged material dispersion theory does not presently allow the use of this method for adequate prediction of initial mixing processes.

Release zone method (227.29(b))

10. Since none of the preceding three methods are feasible until the models are verified (at which time the models will become the generally preferred method), the release zone method of estimating initial mixing must be used in the interim. The liquid and suspended particulate phases of the dredged material may be assumed to be evenly distributed at the end of the 4-hr initial mixing period over a column of water bounded on the surface by the locus of points constantly 100 m from the perimeter of the conveyance engaged in dumping activities, beginning at the first moment in which dumping commences and ending at the last moment (the release zone) and extending to the ocean floor, thermocline, or halocline if one exists, or to a depth of 20 m, whichever is shallower.

11. In order to calculate the initial mixing zone using the release zone method, a few preliminary determinations have to be made. First, one must determine the appropriate depth value: is the thermocline or halocline, the ocean bottom, or 20 m the shallower value? For the following example calculation, it was assumed that the depth of the bottom was 30 m and that there was no density stratification, so 20 m is the appropriate depth value.

12. Next, one must determine the mode of disposal: is the disposal vessel moving or stationary? This example assumes that a disposal vessel 60 m long and 18 m wide was moving at a speed of one m/sec and took 100 sec to release all its volume of dredged material. With these data an initial mixing zone volume V_m may be calculated using the following equation:

$$V_m = \pi(100)^2 d + 200\ w\ d + (200 + w)(u\ t + \ell)\ d \qquad \text{(H1)}$$

where

π = 3.1416

d = appropriate depth value (here 20 m)

w = width of the disposal vessel

ℓ = length of the disposal vessel

u = speed of the disposal vessel in metres per second

t = time in seconds required to empty disposal vessel during discharge.

13. By equation H1, the volume of the example initial mixing zone would be:

$$V_m = (3.1416)(100\ m)^2(20\ m) + 200\ m\ (18\ m)(20\ m) + (200\ m + 18\ m)\ \{(1\ m/sec)(100\ sec) + 60\ m\}\ 20\ m$$

$$V_m = 1{,}397{,}920\ m^3$$

14. If the discharge is instantaneous or from a stationary vessel, equation H1 reduces to:

$$V_m = \pi(100)^2 d + 200\ w\ d + (200 + w)\ \ell\ d \qquad (H2)$$

where the terms are defined as for equation H1.

Application to Limiting Permissible Concentration (LPC)

Liquid phase - water-quality criteria (227.27(a)(1))

15. The LPC of the liquid phase for constituents for which applicable water-quality criteria have been established is that concentration at which none of the constituents of concern will exceed the criteria after allowance for initial mixing. It is possible to predict whether the LPC will be exceeded by the method given in the following example.

16. In this example, the liquid phase was assumed to have a measured concentration of ammonia (the constituent of concern) of 30 mg/ℓ and the disposal site water to have a measured concentration of 0.1 mg/ℓ. The water-quality criterion for the constituent of concern (ammonia) must be determined from the most recent edition of the EPA publication "Quality Criteria for Water." If the water temperature were assumed to be 15°C and the pH of the water to be 8.0, then the water-quality criterion for total ammonia would be found to be 0.75 mg/ℓ.

17. The dilution factor D (the amount by which the liquid phase must be diluted to meet the water-quality criterion) can be determined from the following equation:

$$D = \frac{C_e - C_s}{C_s - C_a} = \frac{30 - 0.75}{0.75 - 0.1} = 45.0 \qquad \text{(H3)}$$

where

C_e = liquid phase concentration of the constituent of interest (ammonia) = 30 mg/ℓ

C_s = water-quality criterion for the constituent of interest = 0.75 mg/ℓ

C_a = ambient disposal site water concentration of constituent of interest = 0.1 mg/ℓ

Note that if the liquid phase concentration C_e is less than the water-quality criterion C_s, no calculation is necessary since no dilution is required to meet the criterion. If the ambient disposal site water concentration C_a is greater than the water-quality criterion C_s, water quality at the disposal site violates the criterion regardless of the proposed disposal operation, and the criterion cannot be achieved by dilution.

18. The volume of the liquid phase V_w can be calculated by equation H4. For purposes of this calculation, the bulk density of the dredged material may be assumed to be 1.5, the particle density 2.6, and the density of the liquid phase 1.0. These approximations should be used unless these parameters have actually been measured for the dredged material in question.

$$V_w = \frac{P_b - P_d}{P_w - P_d}(V_T) = \frac{1.5 - 2.6}{1.0 - 2.6}\ (3058\ m^3) = 2102\ m^3 \qquad \text{(H4)}$$

where

P_b = bulk density (1.5)

P_d = particle density (2.6)

P_w = density of liquid phase (1.0)

V_T = total volume of disposal vessel (here assumed to be 3058 m^3 or 4000 yd^3)

19. The volume of disposal site water necessary to dilute the discharged liquid phase to acceptable levels can be found using the equation:

$$Vol = D\ V_w = 45\ (2102\ m^3) = 94{,}590\ m^3 \qquad \text{(H5)}$$

where

Vol = required volume of disposal site water

D = dilution factor = 45.0 (equation H3)

V_w = the volume of liquid phase in the discharge = 2102 m^3 (equation H4)

20. In this example, ammonia would not exceed the LPC, since the volume of the initial mixing zone (1,397,920 m^3 from paragraph 12 and equation H1) exceeded the volume of disposal site water necessary to dilute the liquid phase to the water-quality criterion for the constituent of interest (94,590 m^3 from paragraph 19 and equation H5). Note that these calculations must be performed for each constituent of concern, since the dilution factor D (equation H3) will be site specific and different for every constituent. The LPC is met only if the applicable water-quality criteria are met by all constituents of concern.

Liquid phase - no water-quality criteria (227.27(a)(2))

21. If bioassays are conducted with the liquid phase, the above approach must be modified, since the constituent(s) causing effects in bioassays cannot be identified, and therefore their concentrations in the liquid phase or disposal site water cannot be measured. The LPC applicable to liquid phase bioassay interpretation is the concentration that, after initial mixing, will not exceed a toxicity threshold of 0.01 of the acutely toxic concentration. The liquid phase bioassay procedures of Appendices D and E require exposure of organisms to various dilutions, expressed in percent of original liquid phase concentration. In order to predict whether the LPC will be exceeded, it is necessary that the dilution expected at the disposal site after initial mixing also be expressed in terms of percent of original liquid phase concentration. This may be done by comparing the dilution calculated by equation H6 to the bioassay results.

22. The volume of the initial mixing zone is calculated as in the example above, using equation H1 or H2 as appropriate. In this case it was found to be 1,397,920 m^3. The volume of the liquid phase contained in the discharge vessel is then calculated by equation H4; in this

example, it was found to be 2102 m^3. The percent of the original liquid phase concentration found at the disposal site after initial mixing C_w may be calculated as:

$$C_w = \frac{V_w}{V_m}(100) = \frac{2102\ m^3}{1{,}397{,}920\ m^3}(100) = 0.15\% \qquad \text{(H6)}$$

where

V_w = volume of liquid phase released in the discharge (equation H4)

V_m = volume of the initial mixing zone (equation H1)

23. According to the solution of equation H6, in this example the original concentration of the liquid phase was diluted by a factor of 667, so that the concentration after initial mixing was only 0.15 percent of the original liquid phase concentration at the instant of release. In order to predict whether this would exceed the LPC, it is necessary to determine whether this concentration is higher or lower than 0.01 (or other factor) of the acutely toxic concentration. This is done by graphically comparing the dilution curve to the time-concentration mortality curve as described in paragraphs 39 through 41 of Appendix D.

Suspended particulate phase (227.27(b))

24. Initial mixing of the suspended particulate phase is estimated in a manner similar to that described in paragraphs 21 through 23 for the liquid phase without water-quality criteria. First the volume of the initial mixing zone is calculated, using equation H1 or H2 as appropriate. In this example the initial mixing zone volume is 1,397,920 m^3.

25. The volume of suspended particulate phase contained in the disposal vessel must then be determined. Since it is impractical to calculate the volume directly, the environmentally protective assumptions are made that all silt and clay-sized particles are contained in the suspended particulate phase and that they would remain in suspension during the 4-hr initial mixing period. If adequate data are available for the operation in question to demonstrate that this assumption is valid, the most accurate estimate of the percent of material that

would remain in suspension should be incorporated in the calculations. The volume of suspended particulate phase in the discharge V_{sp} can be calculated as:

$$V_{sp} = (V_T - V_w) \frac{(P_c + P_s)}{100} \qquad \text{(H7)}$$

where

V_T = total volume of discharge vessel (3058 m^3)

V_w = volume of liquid phase in the discharge (2102 m^3 from equation H4)

P_c = percent clay in the dredged sediment

P_s = percent silt in the dredged sediment

26. In this example, assumed to be from harbor maintenance dredging and to have 50 percent clay and 40 percent silt, the volume of suspended particulate phase in the discharge would be:

$$V_{sp} = (3058\ m^3 - 2102\ m^3) \frac{(40 + 50)}{100} = (956\ m^3)(0.90) = 860\ m^3$$

27. The percent of the original suspended particulate phase concentration found at the disposal site after initial mixing C_{sp} is calculated from a slight modification of equation H6 as:

$$C_{sp} = \frac{V_{sp}}{V_m} (100) = \frac{860\ m^3}{1{,}397{,}920\ m^3} (100) = 0.06\% \qquad \text{(H8)}$$

where

V_{sp} = volume of suspended particulate phase in the discharge (equation H7)

V_m = volume of the initial mixing zone (equation H1)

28. According to the solution of equation H8, the original suspended particulate phase concentration was diluted by a factor of 1667, so that the concentration at the disposal site after initial mixing was only 0.06 percent of the original suspended particulate phase concentration at the instant of release. In order to predict whether this would exceed the LPC, one must determine whether C_{sp} as calculated in the preceding example is higher or lower than 0.01 (or other factor) of

the acutely toxic concentration. This may be done by graphical comparison of the time-concentration mortality curve and the dilution curve, as discussed in paragraphs 39 through 41 of Appendix D.