



Designation: E 1383 – 94a

AMERICAN SOCIETY FOR TESTING AND MATERIALS
1916 Race St. Philadelphia, Pa 19103

Reprinted from the Annual Book of ASTM Standards. Copyright ASTM
If not listed in the current combined index, will appear in the next edition.

Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates¹

This standard is issued under the fixed designation E 1383; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers procedures for obtaining laboratory data to evaluate adverse effects of contaminants associated with whole sediment on freshwater organisms. The methods are designed to assess the toxic effects on invertebrate survival, growth, or reproduction, from short- (for example, 10 days) or long-term tests, in static or flow-through water systems. Sediments to be tested may be collected from field sites or spiked with known compounds in the laboratory. Test procedures are described for (1) *Hyaella azteca*, (2) *Chironomus tentans*, (3) *Chironomus riparius*, (4) *Daphnia sp.* and *Ceriodaphnia sp.*, (5) *Hexagenia spp.*, (6) *Tubifex tubifex*. Methods described in this guide should also be useful for conducting sediment toxicity tests with other aquatic species, although modifications may be necessary.

1.2 Modification of these procedures might be justified by special needs. Results of tests conducted using unusual procedures are not likely to be comparable to results using this guide. Comparison of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment toxicity tests with freshwater organisms.

1.3 The results from field-collected sediments used in toxicity tests to determine a spatial or temporal distribution of sediment toxicity may be reported in terms of the biological effects on survival, growth, or reproduction (see Section 16, Calculation). In addition, these procedures are applicable to most sediments or chemicals added to sediment. Materials either adhering to sediment particles or dissolved in interstitial water can be tested. With appropriate modifications these procedures can be used to conduct sediment toxicity tests when factors such as temperature, dissolved oxygen, pH, and sediment characteristics (for example, particle size, organic carbon content, and total solids) are of interest, or when there is a need to test such materials such as sewage sludge, oils, and particulate matter. These methods might also be useful for conducting bioaccumulation tests.

1.4 Results of toxicity tests with test materials experimentally added to sediments may be reported in terms of an LC50 (median lethal concentration) and sometimes an EC50 (median effect concentration). Results of tests may be reported in terms of an NOEC (no observed effect concen-

tration) and LOEC (lowest observed effect concentration).

1.5 This guide is arranged as follows:

Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Interferences	6
Apparatus	7
Hazards	8
Overlying Water	9
Sediment Characterization	10
Test Organisms	11
Experimental Design	12
Procedure	13
Analytical Methodology	14
Acceptability of Test	15
Calculation	16
Report	17
Keywords	18

Annexes

- A1. *Hyaella azteca* (Amphipoda)
- A2. *Chironomus tentans* (Diptera)
- A3. *Chironomus riparius* (Diptera)
- A4. *Daphnia sp.* and *Ceriodaphnia sp.*
- A5. *Hexagenia spp.*
- A6. *Tubifex tubifex* (Müller 1774)
- A7. *Diporeia spp.*

1.6 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Specific hazard statements are given in Section 8.

2. Referenced Documents

2.1 ASTM Standards:

- D 1129 Terminology Relating to Water²
- D 4387 Classification of Grab Sampling Devices for Collecting Benthic Macroinvertebrates³
- D 4447 Guide for the Disposal of Laboratory Chemicals and Samples³
- E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)⁵
- E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians³

¹ This guide is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.03 on Sediment Toxicology.

Current edition approved July 15, 1994. Published September 1994. Originally published as E 1383 – 90. Last previous edition E 1383 – 94.

² Annual Book of ASTM Standards, Vol 11.01.

³ Annual Book of ASTM Standards, Vol 11.04.

⁴ Annual Book of ASTM Standards, Vol 11.02.

⁵ Annual Book of ASTM Standards, Vol 14.02.

- E 943 Terminology Relating to Biological Effects and Environmental Fate³
- E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses³
- E 1193 Guide for Conducting Renewal Life-Cycle Toxicity Tests with Saltwater Mysids³
- E 1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes³
- E 1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*³
- E 1297 Test Method for Measuring Fast Neutron Reaction Rates by Radioactivation of Niobium⁶
- E 1367 Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods³
- E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing³

3. Terminology

3.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified conditions, unless the purpose of the test requires a different design. “Must” is used only in connection with the factors that directly relate to the acceptability of the test (see Section 15). “Should” is used to state that the specified condition is recommended and ought to be met if possible. Although a violation of one “should” is rarely a serious matter, violation of several will often render the results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean “is (are) allowed to,” “can” is used to mean “is (are) able to,” and “might” is used to mean “could possibly.” Thus, the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can.”

3.2 *Definitions*—For definitions of other terms used in this guide, refer to Guides E 729, E 1023, and E 1241, and Terminologies D 1129 and E 943. For an explanation of units and symbols, refer to Practice E 380.

3.3 *Descriptions of Terms Specific to This Standard:*

3.3.1 *clean*—denotes a sediment or water that does not contain concentrations of test materials which cause apparent stress to the test organisms or reduce their survival.

3.3.2 *concentration*—the ratio of weight or volume of test material(s) to the weight or volume of sediment.

3.3.3 *interstitial water*—the water within a wet sediment that surrounds the sediment particles, expressed as the percent ratio of the weight of the water in the sediment to the weight of the wet sediment.

3.3.4 *overlying water*—the water placed over the whole sediment in the test chamber for the conduct of the toxicity test, and may also include the water used to manipulate the sediments.

3.3.5 *sediment*—a naturally occurring particulate material which has been transported and deposited at the bottom of a body of water, or an experimentally prepared substrate

within which the test organisms can interact.

3.3.6 *spiking*—the experimental addition of a test material such as a chemical or mixture of chemicals, sewage sludge, oil, particulate matter, or highly contaminated sediment to a clean negative control or reference sediment, such that the toxicity of the material added can be determined. After the test material is added, which may involve a solvent carrier, the sediment is thoroughly mixed to evenly distribute the test material throughout the sediment.

3.3.7 *whole sediment*—distinguished from elutriates, and resuspended sediments, in that the whole, intact sediment is used to expose the organisms, not a form or derivative of the sediment.

4. Summary of Guide

4.1 The toxicity of contaminated whole sediments is assessed during continuous exposure of aquatic organisms, using either static or flow-through exposure systems. Sediments tested may either be collected from field sites or spiked with a known compound(s). A negative control sediment or a reference sediment is used to (1) give a measure of the acceptability of the test; (2) provide evidence of the health and relative quality of the test organisms; (3) determine the suitability of the overlying water, test conditions, food, and handling procedures; and (4) provide a basis for interpreting data obtained from the test sediments. A reference sediment is collected from the field in a clean area and represents the test sediments in sediment characteristics (for example, TOC, particles size, and pH). Specified data are obtained to determine the toxic effects on survival, growth, or reproduction, from short- (for example, 10 days) or long-term exposures to aquatic invertebrates.

5. Significance and Use

5.1 Protection of a species requires averting detrimental contaminant-related effects on the survival, growth, reproduction, health, and uses of the individuals of that species (1).⁷ Sediment toxicity tests provide information concerning the bioavailability of contaminants associated with sediments to aquatic organisms. Invertebrates occupy an essential niche in aquatic ecosystems and are an important food source for fish, wildlife, and larger invertebrates. A major change in the availability of invertebrates as either a food source, or as organisms functioning properly in trophic energy transfer and nutrient cycling, could have serious adverse ecological effects on the entire aquatic system.

5.2 Results from sediment toxicity tests might be an important consideration when assessing the hazards of materials on aquatic organisms (see Guide E 1023) or when deriving sediment quality concentrations for aquatic organisms (2).

5.3 Information might also be obtained on accumulation of contaminants associated with sediments by analysis of animal tissues for the contaminant(s) being monitored.

5.4 The sediment toxicity test might be used to determine the temporal or spatial distribution of sediment toxicity. Test

⁶ Annual Book of ASTM Standards, Vol 12.02.

⁷ The boldface numbers in parentheses refer to the list of references at the end of this standard.

methods can be used to detect horizontal and vertical gradients in toxicity.

5.5 Results of sediment toxicity tests with test materials experimentally added to sediments could be used to compare the sensitivities of different species and the toxicity of different test materials, and to study the effects of various environmental factors or results of such tests. Results of sediment toxicity tests are useful for studying biological availability of test materials and structure-activity relationships.

5.6 Results of sediment toxicity tests can be used to predict effects likely to occur with aquatic organisms in field situations as a result of exposure under comparable conditions, except that (1) motile organisms might avoid exposure and (2) toxicity to benthic organisms can be dependent on the sediment physical characteristics, dynamics of equilibrium partitioning, and route of exposure.

5.6.1 Field surveys can be designed to provide either a qualitative reconnaissance of the distribution of sediment toxicity or a quantitative statistical comparison of toxicity among sites.

5.6.2 Sediment toxicity surveys are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic conditions. Statistical correlation can be improved and costs reduced if subsamples for sediment toxicity tests, geochemical analyses, and benthic community structure are taken simultaneously from the same grab of the same site.

5.7 Sediment toxicity tests can be an important tool for making decisions regarding the extent of remedial action needed for contaminated aquatic sites.

6. Interferences

6.1 Limitations to the methods described in this guide might arise and thereby influence sediment toxicity test results and complicate data interpretation. The following factors should be considered when testing whole sediments:

6.1.1 Alteration of field samples in preparation for laboratory testing (for example, sieving),

6.1.1.1 Maintaining the integrity of the sediment environment during its removal, transport, and testing in the laboratory is extremely difficult. The sediment environment is composed of a myriad of microenvironments, redox gradients, and other interacting physiochemical and biological processes. Many of these characteristics influence sediment toxicity and bioavailability to benthic and planktonic organisms, microbial degradation, and chemical sorption. Any disruption of this environment complicates interpretations of treatment effects, causative factors, and in situ comparisons.

6.1.1.2 Sediments tested at temperatures other than that at which they are collected might affect contaminant solubility, partitioning coefficients, and other physical and chemical characteristics.

6.1.2 Interaction between sediment and overlying water and the influences of the ratio of sediment to overlying water,

6.1.3 Interaction among chemicals present in the sediment,

6.1.4 Use of laboratory spiked sediment that might not be

representative of contaminants associated with sediments in the field,

6.1.5 Maintenance of acceptable quality of overlying water,

6.1.6 Addition of food (3) or solvents to the test chambers that might obscure the adverse influence of contaminants associated with sediment and provide an organic substrate for bacterial or fungal growth, and might affect water quality characteristics (4),

6.1.7 Resuspension of sediment during the toxicity test,

6.1.8 Natural geochemical properties of test sediment collected from the field that might not be within the tolerance limits of the test species,

6.1.9 Recovery of test organisms from the sediment,

6.1.10 Field-collected sediments that may contain indigenous organisms including predators, the same or closely related species to that being tested, and microorganisms (for example, bacteria and molds) and algae species that might grow in or on the sediment and test chamber surfaces, and

6.1.11 Test material concentrations that might be reduced in the overlying water in flow-through testing, and compounds such as ammonia that might increase during testing.

6.2 Static tests might not be applicable with materials that are highly volatile or rapidly transform biologically or chemically. The dynamics of test material partitioning between solid and dissolved phases at the start of the test should therefore be considered, especially in relation to assumptions of chemical equilibria.

7. Apparatus

7.1 *Facilities*—The facility should include constant temperature areas for culturing and testing to reduce the possibility of contamination by test materials and other substances, especially volatile compounds. Holding, acclimation, and culture tanks should not be in a room in which toxicity tests are conducted, stock solutions or test solutions are prepared, or equipment is cleaned. Test chambers may be placed in a temperature-controlled recirculating water bath or a constant-temperature area. Air used for aeration should be free of fumes, oil, and water. Filters to remove oil, water, and bacteria are desirable. Air filtration through a 0.22- μ m bacterial filter or other suitable system may be used. The test facility should be well ventilated and free of fumes. Enclosures might be desirable to ventilate test chambers.

7.1.1 If a photoperiod other than continuous light is used, a timing device should be used to provide a light:darkness cycle. A 15 to 30-min transition period (5) when lights go on and off might be desirable to reduce the possibility of test organisms being stressed by instantaneous illumination; a transition period when lights go off might also be desirable.

7.2 *Construction Materials*—Equipment and facilities that contact stock solutions, test solutions, sediment, and overlying water, into which test organisms will be placed, should not contain substances that can be leached or dissolved in amounts that adversely affect the test organisms. In addition, equipment and facilities that contact sediment or water should be chosen to minimize sorption of test materials from water. Glass, Type 316 stainless steel, nylon, high-density polyethylene, polycarbonate, and fluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption. Concrete and rigid

(unplasticized) plastics may be used for holding, acclimation, and culture tanks, and in the water-supply system, but these materials should be soaked, preferably in flowing water, for a week or more before use (6). Cast-iron pipe should probably not be used in freshwater-supply systems because colloidal iron will be added to the overlying water and strainers will be needed to remove rust particles. Copper, brass, lead, galvanized metal, and natural rubber should not contact overlying water or stock solutions before or during the test. Items made of neoprene rubber and other materials not mentioned, should not be used unless it has been shown that their use will not adversely affect survival, growth, or reproduction of the test organisms.

7.3 Water Delivery System—The water delivery system used in flow-through testing can be one of several designs. The system should be capable of delivering water to each replicate test chamber. Several designs of diluter systems are currently in use; Mount and Brungs (7) diluters have been successfully modified for sediment testing, and other diluter systems have also been useful, according to Ingersoll and Nelson (4) and Maki (8). Various metering systems, using different combinations of siphons, pumps, solenoids, valves, etc., have been used successfully to control the flow rates of overlying water.

7.3.1 The metering system should be calibrated before the test by determining the flow rate of the overlying water through each test chamber. The general operation of the metering system should be visually checked daily throughout the conduct of the test. If necessary, the water delivery system should be adjusted during the test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10 %.

7.4 Test Chambers:

7.4.1 In a toxicity test with aquatic organisms, test chambers are defined as the smallest physical units between which there are no water connections. However, screens, cups, etc. may be used to create two or more compartments within each chamber. Therefore, the overlying water can flow from one compartment to another within a test chamber but, by definition, cannot flow from one chamber to another. All test chambers and compartments, if used in a sediment toxicity test, must be identical. For the static tests, cover watchglasses may be used to fit over the top of the test chambers such that an aeration tip is accommodated.

7.4.2 Test chambers may be constructed in several ways of various materials, depending on the experimental design and the contaminants of interest. Clear silicone adhesives, suitable for aquaria, sorb some organic compounds which might be difficult to remove. Therefore, as little adhesive as possible should be in contact with the test solution. If extra beads of adhesive are needed, they should be on the outside of the test chambers rather than on the inside. To leach potentially toxic compounds from the adhesive, all new test chambers constructed using silicone adhesives should be acclimated at least 48 h in overlying water used in the sediment toxicity test.

7.4.3 Species-specific information on test chambers is given in Annexes A1 through A7.

7.5 Cleaning:

7.5.1 Test chambers, water delivery systems, equipment used to prepare and store overlying water, and stock solu-

tions should be cleaned before use. New items should be washed in the following manner: (1) detergent wash, (2) water rinse, (3) water-miscible organic solvent wash, (4) water rinse, (5) acid wash (such as 10 % concentrated hydrochloric acid), and (6) rinsed at least twice with distilled, deionized, or overlying water. Test chambers should be rinsed with overlying water just before use.

7.5.2 Many organic solvents leave a film that is insoluble in water. A dichromate-sulfuric acid cleaning solution can generally be used in place of both the organic solvent and the acid (see Practice E 729), but the solution might attack silicone adhesive and leave chromium residues on glass.

7.5.3 Upon completion of a test, all items to be used again should be immediately emptied of sediment and overlying water (and properly disposed), rinsed with water, cleaned by a procedure appropriate for removing the test material (for example, acid to remove metals and bases; detergent, organic solvent, or activated carbon to remove organic chemicals), and rinsed at least twice with distilled, deionized, or overlying water, in that order.

7.6 Acceptability—Before a toxicity test is conducted in new test facilities, it is desirable to conduct a “non-toxicant” test, in which all test chambers contain a negative control or reference sediment, and overlying water with no added test material. Survival, growth, or reproduction of the test species will demonstrate whether facilities, water, control sediment, and handling techniques are adequate to result in acceptable species-specific control numbers. The magnitude of the within-chamber and between-chamber variance should also be determined.

8. Hazards

8.1 Many substances pose health risks to humans if adequate precautions are not taken. Information on toxicity to humans, recommended handling procedures, and chemical and physical properties of the test material should be studied before a test is begun and made aware to all personnel involved (9–12). Contact with test materials, overlying water, and sediments should be minimized.

8.1.1 Many materials can adversely affect humans if precautions are inadequate. Skin contact with test materials and solutions should be minimized by such means as wearing appropriate protective gloves, laboratory coats, aprons, and safety glasses, and by using dip nets, sieves, or tubes to remove test organisms from overlying water. When handling hazardous sediments the proper handling procedures might include sieving and distributing sediments under a ventilated hood or in an enclosed glove box, enclosing and ventilating the toxicity testing water bath, and use of respirators, aprons, safety glasses, and gloves. Field-collected sediments might contain toxic materials and should be treated with caution to minimize occupational exposure to workers. Worker safety should also be considered when working with spiked sediments containing organics or inorganic contaminants, those that are radio-labeled, and with materials that are, or are suspected of being, carcinogenic (11).

8.2 Careful considerations should be given to those chemicals which might biodegrade, transform to more toxic components, volatilize, oxidize, or photolyze during the test period.

8.3 For tests involving spiked sediments with known test materials, removal or degradation of test material before disposal of stock solutions, overlying water, and sediments is sometimes desirable.

8.4 Health and safety precautions and applicable regulations for disposal of stock solutions, test organisms, sediments, and overlying water should be considered before beginning a test (see Guide D 4447).

8.5 Cleaning of equipment with a volatile solvent such as acetone should be performed only in a well-ventilated area in which no smoking is allowed and no open flame such as a pilot light is present.

8.6 An acidic solution should not be mixed with a hypochlorite solution because hazardous fumes might be produced.

8.7 To prepare dilute acid solutions, concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be performed only in a fume hood.

8.8 Use of ground fault systems and leak detectors is strongly recommended to help prevent electrical shocks.

9. Overlying Water

9.1 *Requirements*—Besides being available in adequate supply, overlying water used in toxicity tests, and water used to hold organisms before testing, should be acceptable to test species and uniform in quality. To be acceptable to the test species, the water must allow satisfactory survival and growth, without showing signs of disease or apparent stress, such as discoloration or unusual behavior.

9.2 Source:

9.2.1 Natural overlying water should be uncontaminated and of constant quality and should meet the following specifications as established in Guide E 729. The values stated help to ensure that test organisms are not apparently stressed during holding, acclimation, and testing, and that test results are not unnecessarily affected by water quality characteristics:

Particulate matter	<5 mg/L
TOC	<5 mg/L
COD	<5 mg/L
Residual chlorine	<11 µg/L

9.2.1.1 A natural overlying water is considered to be of uniform quality if the monthly ranges of the hardness, alkalinity, and specific conductance are less than 10 % of their respective averages and if the monthly range of pH is less than 0.4 unit. Natural overlying waters should be obtained from an uncontaminated well or spring, if possible, or from a surface water source. If surface water is used, the intake should be positioned to minimize fluctuations in quality and the possibility of contamination and maximize the concentration of dissolved oxygen and to help ensure low concentrations of sulfide and iron. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chlorine, or chloramines, and quality is often variable (13). Chlorinated water should not be used for, or in the preparation of, overlying water because residual chlorine and chlorine-produced oxidants are toxic to many aquatic animals (14). Dechlorinated water should only be used as a last resort because dechlorination is often incomplete.

9.2.2 For certain applications the experimental design might require use of water from the test sediment collection site.

9.2.3 Reconstituted water is prepared by adding specified amounts of reagent grade⁸ chemicals to high-quality distilled or deionized water (see Guide E 729). Acceptable water can be prepared using deionization, distillation, or reverse-osmosis units. Conductivity, pH, hardness and alkalinity should be measured on each batch of reconstituted water. If the water is prepared from a surface water, total organic carbon, or chemical oxygen demand should be measured on each batch. Filtration through sand, rock, bag, or depth-type cartridge filters may be used to keep the concentration of particulate matter acceptably low. The reconstituted water should be intensively aerated before use, except that buffered soft fresh waters should be aerated before, but not after, addition of buffers. Problems have been encountered with some species in some fresh reconstituted waters, but these problems can be overcome by aging the reconstituted water for one or more weeks.

9.3 Characterization:

9.3.1 The following items should be measured at least twice each year, and more often if such measurements have not been determined semiannually for at least two years, or if surface water is used: pH, particulate matter, TOC, organophosphorus pesticides, organic chlorine (or organochlorine pesticides plus PCBs), chlorinated phenoxy herbicides, ammonia, cyanide, sulfide, bromide, chloride, fluoride, iodide, nitrate, phosphate, sulfate, calcium, magnesium, sodium, potassium, aluminum, arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, silver, and zinc, hardness, alkalinity, and conductivity (see Guide E 729).

9.3.2 For each method used the detection limit should be below the concentration in the overlying water, or below the lowest concentration that has been shown to adversely affect the test species (14).

9.3.3 Water that might be contaminated with facultative pathogens may be passed through a properly maintained ultraviolet sterilizer (15) equipped with an intensity meter and flow controls or passed through a filter with a pore size of 0.45 µm or less.

9.3.4 Water might need intense aeration using air stones, surface aerators, or column aerators (16–18). Adequate aeration will stabilize pH, bring concentrations of dissolved oxygen and other gases into equilibrium with air, and minimize oxygen demand and concentrations of volatiles. The concentration of dissolved oxygen in water should be between 90 and 100 % saturation (19) to help ensure that dissolved oxygen concentrations are acceptable in test chambers.

10. Sediment Characterization

10.1 *General*—Before the preparation or collection of sediment an approved written procedure should be prepared

⁸ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Anal. Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

for the handling of sediments which might contain unknown quantities of toxic contaminants (see Section 8, Hazards). All sediments should be characterized and at least the following determined: pH, organic carbon content (total organic carbon, TOC) or total volatile sulfides, particle size distribution (percent sand, silt, clay), and percent water content (20, 21). Other analyses on sediments might include biological oxygen demand, chemical oxygen demand, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis. Macrobenthos may be determined by a subsample of the field-collected sediment. Toxicological results might provide information directing a more intensive analysis. Sediment toxicity testing procedures are detailed in Section 13, Procedure.

10.2 Negative Control and Reference Sediment—A negative control sediment or a reference sediment is used to (1) give a measure of the acceptability of the test, (2) provide evidence of the health and relative quality of the test organisms, (3) determine the suitability of the overlying water, test conditions, food, handling procedures, and (4) provide a basis for interpreting data obtained from the test sediments. Every test requires a negative sediment control (sediment known to be nontoxic to, and within the geochemical requirements of, the test species) or a reference sediment. A reference sediment should be collected from the field in a clean area and represent the test sediment in sediment characteristics (for example, TOC, particles size, pH). This provides a site-specific basis for comparison of toxic and nontoxic conditions. The same overlying water, conditions, procedures, and organisms should be used as in the other treatments, except that none of the test material(s) being tested, or contaminated field-collected sediments, is added to the negative control or reference sediment test chambers.

10.2.1 If a field sediment has properties such as grain size and organic content that might exceed the tolerance range of the test species, it is desirable to include a reference sediment for these characteristics.

10.3 Field-Collected Test Sediment:

10.3.1 Collection (see Section 8, Hazards)—A benthic grab or core should be used rather than a dredge to minimize disruption of the sample (see Classification D 4387 and Guides E 1367 and E 1391). If the sediment is obtained with a grab, it is preferable to collect a sediment sample from the upper 2 cm. This operation is facilitated if the grab can be opened from the top so that the undisturbed sediment surface is exposed. The sample should be transferred to a clean (see 7.5) sample container. If the contaminants associated with sediments include compounds that readily photolyze, minimize direct sunlight during collection. All sediment samples should be cooled to $4 \pm 2^\circ\text{C}$ in the field.

10.3.2 Storage—Sediment samples should be stored at $4 \pm 2^\circ\text{C}$ and for no longer than two weeks before the start of the test. Freezing and longer storage might change sediment properties and should be avoided (see Guides E 1367 and E 1391). Sediment may be stored in containers constructed of suitable quality in accordance with 7.2. It is desirable to avoid contact with metals, including stainless steel and brass sieving screens, and some plastics. The samples should be

thoroughly mixed and may be wet-press sieved through a suitably sized sieve to remove large particles and indigenous organisms, especially predators. Sediment may be diluted and mixed in a 1 to 1 ratio with overlying water to facilitate sieving (22) (see Section 6, Interferences).

10.3.3 If the experimental design prescribes not sieving a field-collected sediment, obvious large predators or other large organisms should be removed by using forceps. If sediment is to be collected from multiple field samples and pooled to meet technical objectives, the sediment should be thoroughly homogenized by stirring, or with the aid of a rolling mill, feed mixer, or other suitable apparatus (see Guide E 1391).

10.3.4 Additional samples may be taken from the same grab for other kinds of sediment analyses (see 10.1). Qualitative descriptions of the sediment may include color, texture, presence of macrophytes, animals, tracks, and burrows. Monitoring the odor of sediment samples should be avoided because of hazardous volatile contaminants (see Section 8, Hazards).

10.3.5 The natural geochemical properties of test sediment collected from the field must be within the tolerance limits of the test species. The limits for the test species should be determined experimentally in advance (see 10.2). Controls for such factors as particle size distribution, organic carbon content, pH, etc. should be run if the limits are exceeded in the test sediments (23).

10.4 Laboratory-Spiked Sediment—Test sediment can also be prepared in the laboratory by manipulating the properties of the negative control or the reference sediment. This can include adding chemicals or complex waste mixtures (see 1.4) (24). The toxicity of substances either dissolved in the interstitial water or adsorbed to sediment particles can be determined experimentally.

10.4.1 The test material(s) should be reagent grade⁸ or better, unless a test on formulation commercial product (25) or technical-grade or use-grade material is specifically needed. Before a test is started, the following should be known about the test material: (1) the identity and concentration of major ingredients and impurities, (2) water solubility in test water, (3) estimated toxicity to the test species and to humans, (4) precision and bias of the analytical method at the planned concentration(s) of the test material, if the test concentration(s) are to be measured, and (5) recommended handling and disposal procedures. The toxicity of the test material in sediments may be quite different from the toxicity in water-borne exposures.

10.4.2 Stock Solution(s)—Test material(s) to be tested in sediment should be dissolved in a solvent to form a stock solution that is then added to the sediment. The maximum concentration of the solvent in the sediment should be at a concentration that does not affect the test species. The concentration and stability of the chemical in the stock solution should be determined before beginning the test. If the chemical(s) is subject to photolysis, the stock solution should be shielded from the light both before and during the process of mixing into the sediment. If a solvent other than water is necessary (the preferred solvent is water), it should be one which can be driven off (for example, evaporated), leaving only the test chemical on the sediments. Concentrations of the chemical in the water and sediment should be

monitored before the test begins.

10.4.3 If a solvent other than water is used, both a sediment solvent control and a sediment negative control or reference sediment must be included in the test. The solvent control must contain the highest concentration of solvent present and must use solvent from the same batch used to make the stock solution (see Guide E 729). The same concentration of solvent should be used in all treatments.

10.4.3.1 Triethylene glycol is often a good organic solvent for preparing stock solutions because of its low toxicity to aquatic animals, low volatility, and ability to dissolve many organic chemicals. Other water-miscible organic solvents, such as methanol, ethanol, or acetone may be used, but they might affect total organic carbon levels, introduce toxicity, alter the geochemical properties of the sediment, or stimulate undesirable growths of microorganisms (see Section 6, Interferences). Acetone is highly volatile and might leave the system more readily than methanol or ethanol. A surfactant should not be used in the preparation of a stock solution because it might affect the bioavailability, form, and toxicity of the test material.

10.4.4 If the concentration of solvent is not the same in all test solutions that contain test material, either a solvent test should be conducted to determine whether survival, growth, or reproduction of the test organisms is related to the concentration of the solvent over the range used in the toxicity test, or such a solvent test already conducted using the same overlying water and test species. If survival, growth, or reproduction is found to be related to the concentration of the solvent, a sediment toxicity test with that species in that amount of solvent is unacceptable if any treatment contained a concentration of solvent in that range.

10.4.4.1 If the test contains both a negative control and a solvent control, the survival, growth, or reproduction of the organisms tested in the two controls should be compared (see Guide E 1241). If a statistically significant difference in either survival, growth, or reproduction is detected between the two controls, only the solvent control may be used for meeting the acceptability of the test and as the basis for calculation of results. The negative control might provide additional information on the general health of the organisms tested. If no statistically significant difference is detected, the data from both controls should be used for meeting the acceptability of the test and as the basis for calculation of results (see 9.2.4.3 of Guide E 1241).

10.4.5 *Test Concentration(s) for Laboratory-Spiked Sediments:*

10.4.5.1 If the test is intended to allow calculation of an LC50, the test concentrations should bracket the predicted LC50. The prediction might be based on the results of a test on the same or a similar test material on the same or a similar species. The LC50 of a particular compound may vary depending on physical and chemical sediment characteristics. If a useful prediction is not available, it is desirable to conduct a range-finding test in which the organisms are exposed to a control and three or more concentrations of the test material that differ by a factor of ten.

10.4.5.2 If necessary, concentrations above aqueous solubility can be used, as indigenous organisms are at times exposed to concentrations above solubility in the real world (see Guide E 729).

10.4.5.3 Bulk sediment chemical concentrations might be normalized to factors other than dry weight. For example, concentrations of nonpolar organic compounds might be normalized to sediment organic carbon content and metals normalized to acid volatile sulfides.

10.4.5.4 In some situations (for example, regulatory) it might be necessary to only determine whether a specific concentration of test material is toxic to the test species, or whether the LC50 is above or below a specific concentration. When there is interest in a particular concentration, it might only be necessary to test that concentration and not to determine the LC50.

10.4.6 Addition of test material(s) to sediment may be accomplished using various methods, such as a (1) rolling mill, (2) feed mixer, or (3) hand mixing (see Guide E 1391).

10.4.6.1 Modifications of the mixing techniques might be necessary to allow time for a test material to equilibrate with the sediment. If tests are repeated, mixing conditions such as duration and temperature of mixing, and time of mixing before the test starts, should be kept constant. Care should be taken to ensure that a test material added to sediment is thoroughly and evenly distributed within the sediment. If necessary, subsamples of the sediment within a mixing container can be analyzed to determine the degree of mixing and homogeneity.

11. Test Organisms

11.1 *Species*—Whenever possible and appropriate, tests should be conducted with species listed in the annexes. Use of these species is encouraged to increase comparability of results. The source and type of sediment being tested or the type of test to be implemented might dictate selection of a particular species. The species used should be selected based on availability, sensitivity to a test material(s), and tolerance to ecological conditions such as temperature, grain size, and ease of handling in the laboratory. The species used should be identified using an appropriate taxonomic key.

11.2 *Age*—All organisms should be as uniform as possible in age and size class. The age or size class for a particular test species should be chosen so that sensitivity to test materials is not affected by state of maturity, reproduction, or other intrinsic life-cycle factors (see Annexes A1 through A7).

11.3 *Source*—All organisms in a test must be from the same source. Organisms may be obtained from laboratory cultures; commercial, state or federal institutions; or natural populations from clean areas. Laboratory cultures of test species can provide organisms whose history, age, and quality are known. Local and state agencies might require collecting permits.

11.4 *Quality*—Analysis of the test organisms for the test material(s) is desirable, as it might be present in the environment, and other chemicals to which major exposure might have occurred.

11.5 *Brood Stock*—Brood stock should be cared for properly so as not to be unnecessarily stressed (see Annexes A1 through A7). To maintain organisms in good condition and avoid unnecessary stress, they should not be crowded and should not be subjected to rapid changes in temperature or water quality characteristics.

11.6 *Handling*—Test organisms should be handled as little as possible. When handling is necessary, it should be

done as gently, carefully, and as quickly as possible. Organisms should be introduced into solutions beneath the air-water interface. Any organisms that touch dry surfaces, are dropped, or are injured during handling should be discarded.

12. Experimental Design

12.1 Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers and test organisms per treatment, and water quality characteristics, should be based on the purpose of the test and the type of procedure that is to be used to calculate results (see Section 16, Calculation). A test intended to allow calculation of a specific endpoint such as an LC50 should consist of a negative control sediment, a solvent control(s), a reference sediment, and several test sediments (see Section 10, Sediment Characterization).

12.2 The object of a qualitative reconnaissance survey is to identify sites of toxic conditions that warrant further study. It is often conducted in areas where little is known about contamination patterns. To allow for maximum spatial coverage, the survey design might include only one sample from each site. The lack of replication usually precludes statistical comparisons, but identification of samples for further study is possible, where survival, growth, or reproduction differ from the negative control or reference sediment. A useful summary of field sampling design is presented by Green (26).

12.2.1 The object of a quantitative statistical comparison is to test for statistically significant differences in effects (see 13.12) among negative control or reference sediments and test sediments from several sites. The number of replicates needed per site is a function of the need for sensitivity or power. Replicates (for example, separate samples from different grabs taken at the same site) should be taken at each site in the survey. Separate subsamples from the same grab might be used to test for within-grab variability, or split samples of composited sediment from one or more grabs might be used for comparisons of test procedures (such as comparative sensitivity among test species), but these subsamples should not be considered to be true replicates for statistical comparisons among sites.

12.2.2 Site locations might be distributed along a known pollution gradient, in relation to the boundary of a disposal site, or at sites identified as being toxic in a reconnaissance survey. Comparisons can be made in both space and time (see Section 16, Calculation). In pre-dredging studies, a sampling design can be prepared to assess the toxicity of samples representative of the project area to be dredged. Such a design should include subsampling cores taken to the project depth.

12.3 *Laboratory Experiments*—The primary focus of the physical and experimental test design, and statistical analysis of the data, is the experimental unit, which is defined as the smallest physical entity to which treatments can be independently assigned (27). Because overlying water or air cannot flow from one test chamber to another the test chamber is the experimental unit (see 7.4). As the number of test chambers per treatment increases, the number of degrees of freedom increases, and therefore the width of the confidence interval on a point estimate, such as an LC50, decreases, and the power of a significance test increases (see

Section 16, Calculation). Because of factors that might affect results within test chambers and results of the test, (1) all test chambers should be treated as similarly as possible, such as temperature and lighting (unless these are the variables tested), and (2) each test chamber, including replicate test chambers, must be physically treated as a separate entity. Treatments must be randomly assigned to individual test chamber locations. Assignment of test organisms to test chambers must be randomized.

13. Procedure

13.1 *Sediment into Test Chambers*—The day before the toxicity test is started (Day -1) each test sediment, reference sediment, and negative control sediment should be mixed and a sample added to the test chambers (4, 24, 28). Sediment depth in the test chamber is dependent on the experimental design and the test species (see Annexes A1 through A7 and 6.1.2). Each test chamber and replicates must contain the same amount of sediment, determined either by volume or weight.

13.1.1 The sediment aliquot in each test chamber should be settled by smoothing with a utensil constructed of a suitable material (see 7.2). If beakers are used, bubbles can be removed by either tapping the test chamber against the palm of the hand or by displacement of bubbles with the utensil. After the sediment is placed in the test chambers, overlying water should be added. The overlying water should be gently poured along the side of the test chamber to prevent resuspension of the sediment.

13.2 *Static Testing*—Overlying water should be added to the test chambers at the volume specified by the experimental design. Watchglasses should be used to cover the test chambers and overlying water gently aerated. Aeration can be provided to each test chamber through a 1-mL glass pipet that extends between the beaker spout and the watchglass cover to a depth not closer than 2 cm from the sediment surface. Air should be bubbled into the test chambers at a rate that does not cause turbulence or disturb the sediment surface. To allow any suspended sediments to settle, the test organisms should not be introduced into the test system for between 12 and 24 h. Water quality characteristics should be measured prior to the addition of the test organisms (see 13.11).

13.2.1 Water lost to evaporation or splattering should be replaced as needed with temperature acclimated deionized water or overlying water. The water quality of the overlying water in static sediment toxicity tests (water hardness, alkalinity, total dissolved solids, and dissolved oxygen) might be altered by the presence of sediment (4) or by the addition of food to the test chamber (3). These changes in water quality characteristics might influence the availability of contaminants to the test organisms (see Section 6, Interferences).

13.3 *Flow-Through Testing*—The water-delivery system should be turned on before a test is started to verify that the system is functioning properly. The water flow to each test chamber should not differ by more than 10 % (see 7.3.1). The total volume flow per hour for continuous flow diluters should be recorded.

13.3.1 After the sediment has been added (Day -1), overlying water is added to the test chambers (see 13.2). After aliquots are removed for water quality determinations (Day

0), overlying water flow is started prior to the addition of the test organisms and food (4).

13.4 Duration of Test—The test begins when test organisms are first placed in the test chambers (Day 0) and continues for the duration specified in the experimental design for a specific test organism (see Annexes A1 through A7).

13.5 Dissolved Oxygen—The dissolved oxygen concentration in each test chamber should be measured in at least one test chamber in each treatment (1) at the beginning and end of the test and at least weekly (if possible) during the test, (2) whenever there is an interruption of the flow of air (static tests) or water (flow-through tests), and (3) whenever the behavior of the test organisms indicates that the dissolved oxygen concentration might be too low (for example, emergence from the sediment). A measured dissolved oxygen concentration should be $>40\%$ and $\leq 100\%$ saturation (see 12.4.2 of Guide E 729).

13.6 Overlying Water Quality Measurements—Conductivity, hardness, pH, and alkalinity should be measured in all treatments at the beginning and end of a short-term test, and at least weekly during a long-term test, using appropriate ASTM standards when possible.

13.7 Temperature—Test temperature depends upon the species used (see Annexes A1 through A7). Other temperatures may be used to study the effect of temperature on survival, growth, or reproduction of test organisms, and contaminant-related properties (for example, bioavailability). The daily mean test temperature must be within $\pm 1^\circ\text{C}$ of the desired temperature. The instantaneous temperature must always be within $\pm 3^\circ\text{C}$ of the desired temperature.

13.8 Feeding—Recommended food, ration, method, and frequency of feeding test organisms are contained in Annexes A1 through A7. The food used should be analyzed for the test material and other possible contaminants. A batch of food may be used if it will support normal function. Detailed records on feeding rates and appearance of the sediment should be made daily.

13.9 Debris—Any floating debris may be skimmed from the test chambers before test organisms are added. This can be accomplished with a piece of fine nylon screen or other suitable material. If more than 0.1 g of floating debris is removed, an analysis should be performed to determine the amount of chemical removed from the system (25).

13.10 Light—For sediment toxicity tests various light:darkness regimes can be used depending on the species being tested (see Annexes A1 through A7) and various experimental designs.

13.11 Acclimation—Test organisms should be acclimated if they are cultured in water different from the overlying water or temperature (4) (see Annexes A1 through A7).

13.12 Biological Data—Effects indicating toxicity of test sediment include mortality and sublethal effects on growth, maturation, behavior, and reproduction. Test chambers should be observed at least daily. At the end of the exposure period, recovery of the test organisms from sediments should be accomplished following the methods outlined for each species (see Annexes A1 through A7).

13.13 Other Measurements:

13.13.1 Field Sediment—Sediment samples should be collected from the same grab for analysis of sediment

physical and chemical characterizations. A separate sample for benthic faunal analyses may be desirable (see Classification D 4387).

13.13.2 Laboratory-Spiked Sediments—At the beginning and at the end of the experiment, measurement of the concentration of the test material(s) in both stock solutions and sediment is desirable. To monitor changes in sediment or interstitial water chemistry during the course of the experiment, separate sediment chemistry chambers should be set up and sampled at the start and end of the experiment. It is not necessary to add test organisms to these chambers at the beginning of the test, but for later sampling, test organisms should be added after the initial sample is taken.

13.13.2.1 Concentration of test material(s) in overlying water, interstitial water, and sediment should be measured at several concentrations and as often as practical during the test. If possible, the concentration of the test material in overlying water, interstitial water, and sediments should be measured at the start and end of the test. Measurement of test material(s) degradation products might also be desirable.

13.13.2.2 Measurement of test material(s) concentration in water can be accomplished by pipeting water samples from a point midway between top, bottom, and sides of the test chamber. Overlying water samples should not contain any surface scum, any material from the sides of the test chamber, or any sediment.

13.13.2.3 Measurement of test material(s) concentration in sediment at the end of a test can be taken by siphoning the overlying water without disturbing the surface of the sediment, then removing appropriate aliquots of the sediment for chemical analysis.

13.13.2.4 Interstitial water can be sampled by using the water that (1) comes to the surface in a mixing apparatus, (2) is on the surface of the sediment after it settles, (3) is separated from the sediment particles by centrifuging a sediment sample, (4) is filtered through an apparatus to extract interstitial water, (5) has been pressed out of the sediment, or (6) by using an interstitial water sampler. Care should be taken to ensure that contaminants do not transform, degrade, or volatilize during the interstitial water sample preparation (see Guide E 1391).

14. Analytical Methodology

14.1 Chemical and physical data should be obtained using appropriate ASTM standards whenever possible. For those measurements for which ASTM standards do not exist or are not sensitive enough, methods should be obtained from other reliable sources (29).

14.2 Concentrations should be measured for contaminants in bulk sediment, test material(s) in the interstitial water, test material(s) in the overlying water, and test material(s) in the stock solution. In addition, measurement of either the apparent dissolved or undissolved substances of the test material(s) is desirable. The apparent dissolved material is defined and determined as that which passes through a $0.45\text{-}\mu\text{m}$ membrane filter.

14.2.1 If samples of overlying water from test chambers, stock solutions, test sediment, or interstitial water are not to be analyzed immediately, they should be handled and stored appropriately (30) (see Section 10, Sediment Characterization).

14.3 Methods used to analyze food or test organisms

should be obtained from appropriate sources (31).

14.4 The precision and bias of each analytical method used should be determined in an appropriate matrix: that is, sediment, water, tissue. When appropriate, reagent blanks, recoveries, and standards should be included when samples are analyzed.

15. Acceptability of Test

15.1 A sediment toxicity test should be considered unacceptable if one or more of the following occurred, except, for example, if temperature was measured numerous times, a deviation of more than 3°C (see 13.6) in any one measurement might be inconsequential. However, if temperature was measured only a minimal number of times, one deviation of more than 3°C might indicate that more deviations would have been found if the temperature had been measured more often.

15.1.1 All test chambers (and compartments) were not identical (see 7.4.1 and 12.3),

15.1.2 The overlying water was not acceptable to the test organisms (see 9.1),

15.1.3 Test organisms were not acclimated to the appropriate overlying water or temperature (if they were cultured in water different from the overlying water or temperature),

15.1.4 The natural geochemical properties of test sediment collected from the field was not within the tolerance limits of the test species (see 10.3.5),

15.1.5 Appropriate negative and solvent controls, or reference sediment, were not included in the test (see 10.4.3),

15.1.6 The concentration of solvent in the range used affected survival, growth, or reproduction of the test organisms (see 10.4.4),

15.1.7 All animals in the test population were not obtained from the same source, were not all of the same species, or were not of acceptable quality (see 11.3),

15.1.8 Treatments were not randomly assigned to individual test chamber locations and the individual test organisms were not impartially or randomly assigned to test chambers or compartments (see 12.3),

15.1.9 Each test chamber did not contain the same amount of sediment, determined either by volume or by weight,

15.1.10 Temperature, dissolved oxygen, and concentration of test material were not measured, or were not within the acceptable range (see 13.7 and Annexes A1 through A7),

15.1.11 The negative control or reference sediment organisms did not survive, grow, or reproduce as required for the test species (see Annexes A1 through A7), or

15.1.12 Average survival in any negative control chamber was less than acceptable limits (see Annexes A1 through A7).

16. Calculation

16.1 The calculation procedure(s) and interpretation of the results should be appropriate to the experimental design. Procedures used to calculate results of toxicity tests can be divided into two categories: those that test hypotheses and those that provide point estimates. No procedure should be used without careful consideration of the advantages and disadvantages of various alternative procedures, and appropriate preliminary tests, such as those for outliers and for heterogeneity.

16.2 For each set of data, the LC50 or EC50 and its 95 % confidence limits should be calculated (when appropriate) on the basis of (1) the measured initial concentrations of test material, if available, or the calculated initial concentrations for static tests, and (2) the average measured concentrations of test material, if available, or the calculated average concentrations for flow-through tests. If other LC or ECs are calculated, their 95 % confidence limits should also be calculated (see Guide E 729).

16.3 Most toxicity tests produce quantal data, that is, counts of the number of responses in two mutually exclusive categories, such as alive or dead. A variety of methods (32) can be used to calculate an LC50 or EC50 and 95 % confidence limits from a set of quantal data that is binomially distributed and contains two or more concentrations at which the percent dead or effected is between 0 and 100, but the most widely used are the probit, moving average, Spearman-Kärber and Litchfield-Wilcoxon methods. The method used should appropriately take into account the number of test organisms per chamber. The binomial test can also be used to obtain statistically sound information about the LC50 or EC50 even when less than two concentrations kill or affect between 0 and 100 %. The binomial test provides a range within which the LC50 or EC50 should lie.

16.4 When samples from field sites are independently replicated, the sites effects can be statistically compared by *t*-tests, analysis of variance (ANOVA), or regression-type analysis. Analysis of variance is used to determine whether any of the observed differences among the concentrations (or samples) are statistically significant. This is a test of the null hypothesis that no differences exist in the effects at all of the concentrations (or samples) and at the control. If the *F*-test is not statistically significant ($P > 0.05$), it can be concluded that the effects observed in the test material treatments (or field sites) were not large enough to be detected as statistically significant by the experimental design and hypothesis test used. Nonrejection does not mean that the null hypothesis is true. The no-observed-effect concentration (NOEC) based on this end point is then taken to be the highest test concentration tested (33, 34). The amount of effect that occurred at this concentration should be considered.

16.4.1 All exposure concentration effects (or field sites) can be compared with the control effects by using mean separation techniques such as those explained by Chew (35) orthogonal contrasts, Fisher's methods, Dunnett's procedure, or Williams' method. The lowest concentration for which the difference in observed effect exceeds the statistical significant difference is defined as the LOEC for that end point. The highest concentration for which the difference in effect is not greater than the statistical significant difference is defined as the NOEC for that end point (33).

17. Report

17.1 The record of the results of an acceptable sediment toxicity test should include the following information either directly or by reference to available documents:

17.1.1 Name of test and investigator(s), name and location of laboratory, and dates of start and end of test,

17.1.2 Source of negative control, reference or test sediment, method for collection, handling, shipping, storage, and disposal of sediment,