CHAPTER 5 Sediment Manipulations

Manipulation of sediments in the laboratory is often required to achieve certain desired characteristics or forms of material for toxicity testing and chemical analysis. As all manipulation procedures alter some qualities of field samples, it is critical to evaluate the effect that these changes might have on the study objective and on each critical measurement endpoint. Therefore, all procedures used to prepare sediment samples should be explicitly described in the study plan and fully documented. Generally, manipulation procedures should be designed to maintain sample representativeness in terms of toxicity and chemistry by minimizing procedural artifacts. Under certain programs, some analytical procedures and toxicity test protocols necessitate specific manipulations (e.g., seawater or solvent extractions for effluent toxicity tests, USEPA/ACOE, 1991, 1998). The reader should always consult and follow any program or test-specific guidance.

This chapter discusses methods for several common manipulations performed in the laboratory including sieving, spiking, organic carbon modification and formulated sediments, sediment dilution, and elutriate preparation. Other sediment manipulations, such as salinity adjustments or pre-treatment of sediment ammonia or sulfides (often done in conjunction with toxicity testing in certain regulatory programs) are not discussed in this manual as these are well documented elsewhere (e.g., PSEP, 1995; USEPA/ACOE, 1998). The reader should consult these references for further information on these procedures. Figure 5-1 presents a flowchart summarizing the laboratory manipulations discussed in this section, illustrating important issues to be considered for each manipulation.

5.1 Sieving

In general, sieving is not recommended because it can substantially change the physicochemical characteristics of the sediment sample. For example, wet sieving of sediment through fine mesh (≤500 µm openings) has been shown to result in decreased percent total organic carbon and decreased concentrations of total PCBs, which might have been associated with fine suspended organic matter lost during the sieving process (Day et al., 1995). Sieving can also disrupt the natural chemical equilibrium by homogenizing or otherwise changing the biological activity within the sediment (Environment Canada, 1994).

In some cases, however, sieving might be necessary to remove indigenous organisms, which can interfere with subsequent toxicity testing and confound

Checklist

Sediment samples are sieved for the following reasons:

- ✓ To remove unrepresentative material, such as shells, stones, trash, and twigs.
- ✓ To increase homogeneity and replicability of samples.
- ✓ To remove indigenous organisms prior to toxicity testing.
- ✓ To facilitate organism counting, sediment handling, and subsampling.
- ✓ To examine the effects of particle size on toxicity, bioavailability, or contaminant partitioning (ASTM, 2000a).

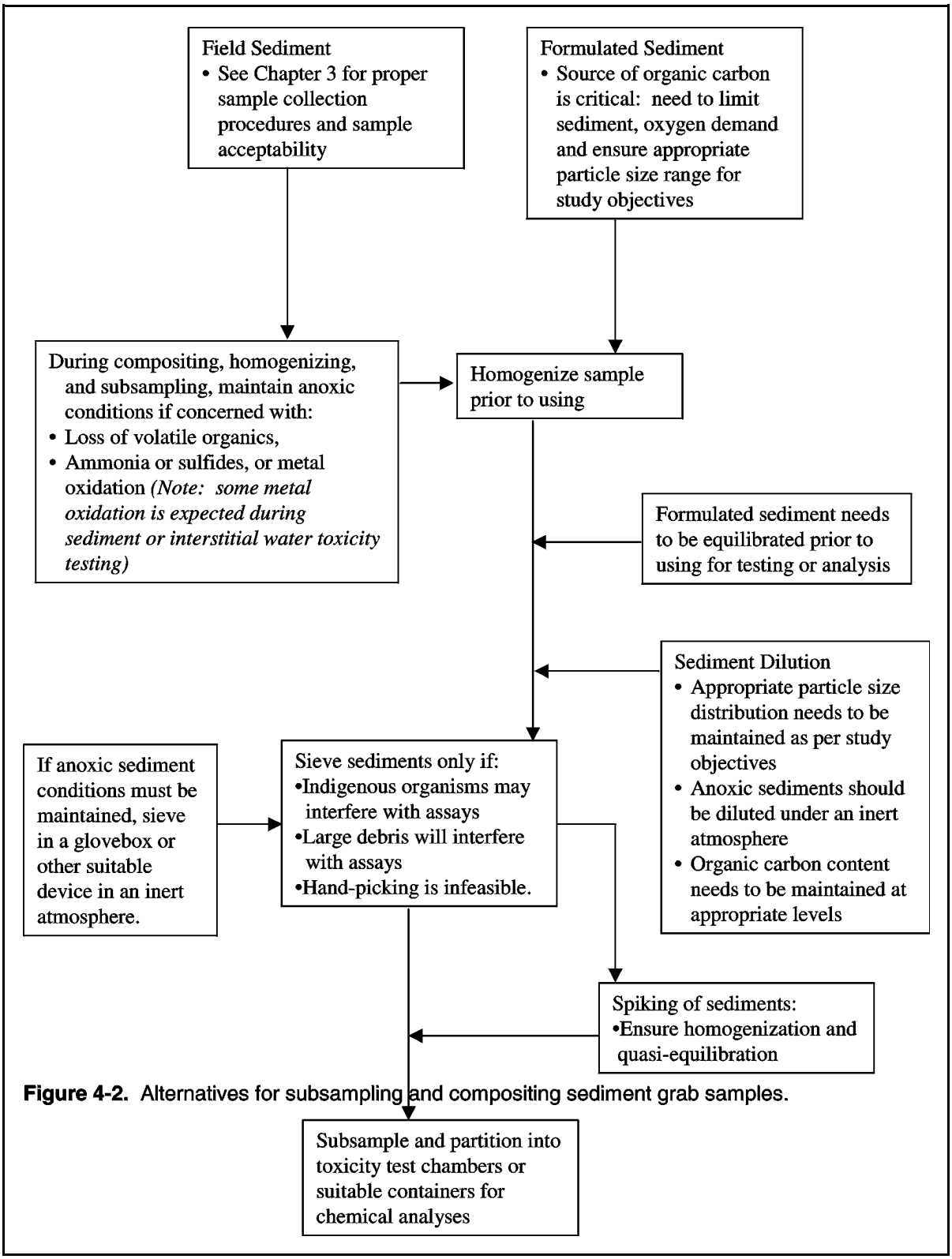


Figure 4-2. Alternatives for subsampling and compositing sediment grab samples.

Figure 5-1. Flowchart depicting relationships between common sediment manipulations including important considerations.

interpretations of analytical results (USEPA, 1994; 2000d; ASTM, 2000e). Indigenous organisms can be problematic in toxicity testing because they might be similar in appearance to test organisms or they might prey on the test organisms.

If sieving is performed, it should be done for all samples to be tested, including control and reference sediments if the objective of the study is to compare results among stations (ASTM, 2000a). It might be desirable to obtain certain measurements (e.g., dissolved and total organic carbon, acid volatile sulfide [AVS], and simultaneously extracted metals [SEM]) both before and after manipulation, to document changes associated with sieving (USEPA, 2000d). In addition, it might be desirable to document the effect of sieving on the sediment sample by conducting comparative toxicity tests using sieved and unsieved sediment (Environment Canada, 1994).

Recommendation Box #1
Should sediment be sieved prior to analyses?

- In general, sieving is not recommended because it can substantially change the physicochemical characteristics of the sediment sample. However, sieving might be necessary in preparing samples for some sediment toxicity testing (i.e., marine amphipod tests; USEPA, 1994).
- Unwanted materials (e.g., large particles and indigenous organisms) can be removed from the sediment sample using forceps as a preferred alternative to sieving.

5.1.1 Sieving Methods

Press Sieving

If sieving is necessary, press sieving is the preferred method. In this method, sediment particles are hand-pressed through a sieve using chemically inert paddles (Giesy et al., 1990; Johns et al., 1991). Matter retained by the screen, such as organisms, shell fragments, gravel, and debris, should be recorded in a log book and discarded (USEPA/ACOE, 1991). Samples with high debris, vegetation, or clay content might be difficult to press through a single sieve with a mesh size less than 1 mm; such samples might need to be pressed through a series of sieves with progressively smaller openings. Water should not be added to sediment when press sieving, as this could result in changes in contaminant concentration and bioavailability. Samples that are going to be used for both chemical analysis and toxicity tests should be sieved together, homogenized, and then split for their respective analyses.

Wet Sieving

If sediments cannot be press sieved without the addition of pressure, wet sieving might be required, however, this type of sieving increases the likelihood of contaminant loss. Wet sieving involves swirling sediment particles within a sieve using water to facilitate the mechanical separation of smaller from larger particles. A slurry made with water that has separated from the sediment during storage or transport might be sufficient to wash particles through the sieve. Wet samples that might have settled during transit should be stirred to incorporate as much field water as possible. In some cases, addition of a small volume of running site or deionized water might be required (ASTM,

Photo courtesy of Allen Burton

Sieving a sediment sample for toxicity testing

2000a). Mechanical shakers or stirring with a nylon brush can also facilitate wet sieving (Mudroch and MacKnight, 1994).

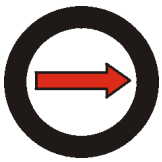
Recommended Sieves

In general, smaller mesh sieves are preferred to reduce loss of fines. Stainless steel, brass, or plastic woven polymer sieves (e.g., polyethylene, polypropylene, nylon, and Teflon) with mesh sizes that vary from 0.24 to 2.0 mm have been used to sieve sediment for toxicity tests (Keilty et al., 1988a;b; Giesy et al., 1990; Lydy et al., 1990; Stemmer et al., 1990a;b; Johns et al., 1991; Landrum and Faust, 1991). Non-metallic sieves are preferred if metals are of interest. Stainless steel sieves are acceptable if organic compounds are of interest. Stainless steel (provided the mesh is not soldered or welded to the frame), nylon, or Nitex-type plastic sieves are recommended when other inorganic constituents are of concern or are to be analyzed (ASTM, 2000a; PSEP, 1995).

Considerations

The mesh type and size should be chosen based on the following considerations...

- ! The type of toxicity test and test organisms to be used.
- ! Potential predators and/or competitors present in the sample.
- ! Potential adsorption or contamination of the chemical of interest due to sieving.
- ! The nature of the sample, including its particle size distribution, volume, and size of debris.



Recommendation Box #2

What type of sieve should be used?

- ➡ Stainless steel or brass sieves are not recommended when metals are a concern or are analyzed (ASTM, 2000a).
- ➡ Smaller mesh sieves (≤2.0 mm mesh openings) are recommended to reduce loss of fine particle sizes.
- ➡ Nylon or nitex mesh sieves are recommended for inorganics analyses (e.g., metals).

Generally, sieving through a 10-mesh (2-mm openings) sieve is acceptable as a basis to discriminate between sediment and other materials (ASTM, 2000a). For toxicity testing, the most frequently used mesh size is 1.0 mm (Environment Canada, 1994), which will remove most adult amphipods. However, a mesh of 0.25 mm might be needed to remove immature amphipods and most macrofauna (Landrum et al., 1992; Robinson et al., 1988; Day et al., 1995). In marine sediments, sieves with a mesh size of 0.5 mm are effective in removing most of the immature amphipods (Swartz et al., 1990; PSEP, 1995).

5.1.2 Alternatives to Sieving

Unwanted materials (e.g., large particles, trash, and indigenous organisms), can be removed from the sediment sample using forceps, prior to or, as an alternative to, sieving. If anerobic integrity of the sample is not a concern, the sediment could be spread on a sorting tray made of cleaned, chemically-inert material, and should be hand-picked with forceps. A stereomicroscope or magnifying lens might facilitate the process, or may be used to determine if sieving is necessary. Hand-picking is

preferable to sieving because it is less disruptive, but it typically is not practical for large volumes of sediment. Of course, this process oxidizes the sediment and might alter contaminant bioavailability.

Autoclaving, freezing, and gamma irradiation of sediments are alternatives to physical removal for inhibiting endemic biological activity in field-collected sediments. These are not generally recommended procedures. Each method has unique effects on the physicochemical and biological characteristics of the sediment, and a careful evaluation with respect to the study objectives is warranted when these methods are considered.

Considerations

In preparing formulated sediments, the following should be noted...

- ! Specific material sources should be carefully selected, as characteristics can vary significantly among product types. A number of suppliers of various sediment components are listed in ASTM (2000a) and USEPA (2000d).
- ! A critical component of formulated sediments is the source of organic carbon. It is not clear that any one source of organic carbon is routinely superior to another. Alpha cellulose appears to be a promising carbon source for formulated sediments.
- ! A variety of formulations have been used successfully in sediment toxicity testing (see ASTM [2000a] and USEPA [2000d]). At this time, no one formulation appears to be universally better than others.

5.2 Formulated Sediment and Organic Carbon Modification

5.2.1 General Considerations

Formulated sediments (also called reconstituted, artificial, or synthetic sediments) are mixtures of materials that mimic the physical components of natural sediments. While they have not been used routinely, formulated sediments potentially offer advantages over natural sediments for use in chemical fate and biological effects testing.

Formulated sediments also have limitations, however. They do not possess the natural microbial, meiofaunal, and macrofaunal communities or the complex organic and inorganic gradients prevalent in natural sediments. The lack of biological activity, diagenesis, and oxidation-reduction (redox) potential gradients undoubtedly alters some sorption and desorption properties, which

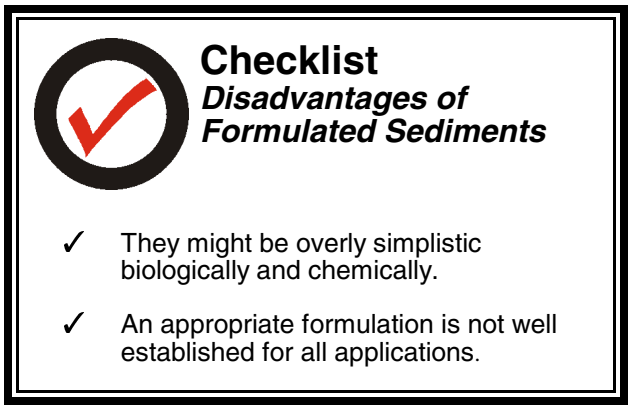
Checklist

Advantages of Formulated Sediments

- ✓ They provide a consistent, reproducible medium which facilitates comparisons between different sets of tests.
- ✓ Eliminates interferences caused by the presence of indigenous organisms.
- ✓ Components of the formulated sediment can be altered to measure the effect of certain physicochemical characteristics on chemical fate and bioavailability.
- ✓ They are useful in spiking experiments to obtain effect concentrations for chemicals.

might in turn alter contaminant fate and effects. The current lack of understanding of physicochemical controls on bioavailability in different sediment environments precludes broad-scale use of formulated sediments in definitive ecological risk assessments.

A formulated sediment should: (1) support the survival, growth, or reproduction of a variety of benthic invertebrates, (2) provide consistent acceptable biological endpoints for a variety of species, and (3) be composed of materials that have consistent characteristics (USEPA, 2000d; ASTM, 2000a). Characteristics should include: (1) consistency of materials from batch to batch, (2) contaminant concentrations below concentrations of concern, and (3) availability to all individuals and facilities (Kemble et al., 1999). Physicochemical characteristics that might be considered when evaluating the appropriateness of a sediment formulation include percent sand/clay/silt, organic carbon content, cation exchange capacity (CEC), redox potential, pH, and carbon:nitrogen:phosphorous ratios (USEPA, 2000d; ASTM, 2000a).



5.2.2 Sediment Sources

The specific material source should be carefully selected, as characteristics can vary significantly among product types. For example, USEPA (2000d) found that for three different sources of kaolinite clay, the percentage of clay ranged from 56.5 to 88.5%, depending on individual product specifications. There are a number of suppliers of various sediment components (see USEPA, 2000d).

A critical component of formulated sediments is the source of organic carbon. It is not clear that any one source of organic carbon is routinely superior to another source.

5.2.3 Organic Carbon Modification

Organic carbon content of natural as well as formulated sediments can be modified to assess the effect on contaminant fate and bioavailability. Many studies employ sediment carbon modifications because total organic carbon (TOC) content has been shown to be a major determinant of nonionic organic chemical bioavailability (DiToro et al., 1991; DeWitt et al., 1992; and Kosian et al., 1999). While TOC modifications might be necessary to achieve study objectives, it should be recognized that organic carbon manipulations can change the particle composition and size distribution, thereby potentially affecting contaminant equilibrium. Thus, results from such experiments should be interpreted with care. Also, the sample needs to be equilibrated (see Section 5.3.3) following addition of the new source of organic carbon, prior to conducting analyses.

Many recipes have used peat as the source of organic carbon, however, the quality and characteristics of peat moss can vary from bag to bag. Other sources of organic carbon include humus, potting soil, maple leaves, composted cow manure, rabbit chow, cereal leaves, chlorella, trout chow, Tetramin®, Tetrafin®, and alpha cellulose. Of these, only peat, humus, potting soil, composted cow manure, and alpha cellulose have been used successfully in sediment testing without fouling the overlying water;

other sources have caused dissolved oxygen concentrations to fall to unacceptable levels (Kemble et al., 1999).

More about organic carbon modification:

Five studies compared organic carbon sources in formulated sediments. A study of 31 different organic carbon recipes by Environment Canada (1995) compared effects on sediment homogeneity, density, and turbidity. Cerophyll and trout chow were selected as the optimal organic carbon sources with high clay (kaolin at 50 or 75% total concentration) and fine sand.

Ribeiro et al. (1994) recommended use of synthetic alpha-cellulose as a carbon source amended with humic acid. This compound has since been tested by Kemble et al (1999), Sawyer and Burton (1994), and Fleming and Nixon (1996). Ribeiro et al. (1994) found that sorption was dependent on the amount of organic carbon present. Kemble et al. (1999) found that growth and survival of *Chironomus tentans* and *Hyalella azteca* was better in 10% than in 2% alpha-cellulose. Both alpha-cellulose and conditioned red maple leaves were found to be suitable as organic carbon amendments for reference toxicant testing with *Hyallela azteca* (96 hr) when spiked with cadmium, zinc, or anthracene (Sawyer and Burton, 1994).

Use of alpha cellulose as a carbon source for sediment-spiking studies has not been adequately evaluated, but it appears to be promising. Alpha cellulose is a consistent source of organic carbon that is relatively biologically inactive and low in concentrations of chemicals of concern. Furthermore, Kemble et al. (1999) reported that conditioning of formulated sediment was not necessary when alpha cellulose was used as a carbon source for a negative control sediment. Compared with other sources of organic carbon, alpha cellulose is highly polymerized and would not serve as a food source, but rather would serve to add texture or provide a partitioning compartment for chemicals.

Reductions in organic carbon content have been achieved by diluting sediment with clean sand (See Section 5.4; Clark et al., 1986; Clark et al., 1987; Tatem, 1986; Knezovich and Harrison, 1988). However, this can change sediment characteristics resulting in non-linear responses in toxicity (Nelson et al., 1993). Combustion has also been used to remove fractions of organic carbon (Adams et al., 1985; IJC, 1988). However, this method results in substantial modification of the sediment characteristics, including oxidization of some inorganic components.

The ratio of carbon to nitrogen to phosphorous might be an important parameter to consider when selecting an organic carbon source. This ratio can vary widely among carbon sources (ASTM, 2000a; USEPA, 2000d). For example, carbon can range from 30 to 47%, nitrogen from 0.7 to 45 mg/g, and phosphorous from below detection limits to 11 µg/g for several different carbon sources (USEPA, 2000d).

A variety of formulations have been used successfully in sediment toxicity testing (see ASTM, 2000a and USEPA, 2000d). At this time, no one formulation appears to be universally better than others.

5.3 Spiking

Spiking involves adding one or more chemicals to sediment for either experimental or quality control purposes. Spiking environmental samples is used to document recoveries of an analyte and thereby analytical bias. Spiked sediments are used in toxicity tests to determine effects of material(s) on test species. Spiking tests can also provide information concerning chemical interactions and transformation rates. The design of spiking experiments, and interpretation of results, should always consider the ability of the sediment to sequester contaminants, recognizing that this governs many chemical and biological processes (O'Donnel et al., 1985; Stemmer et al., 1990a;b; ASTM, 2000a;

Northcott and Jones, 2000). In preparation for toxicity and bioaccumulation tests, references regarding the choice of test concentrations should be consulted (USEPA, 2000d; ASTM, 2000a; Environment Canada, 1995). Program specific guidance documents should also be consulted as appropriate.

Several issues regarding sediment spiking are addressed in this section. First, several methods have been used to spike sediments but the appropriate method needs to be selected carefully depending on the type of material being spiked (e.g., soluble in water or not), its physical-chemical form, and objectives of the particular study. Second, spiked material should be uniformly distributed throughout the sediment. Otherwise, analyses or toxicity tests are likely to yield highly variable results, depending on the concentration of spiked material present. Third, the spiked material needs to be at equilibrium between the sediment and the interstitial water to ensure that all relevant exposure phases are appropriately considered in chemical analyses or toxicity testing. The time it takes to reach this equilibrium is a critical factor that needs to be considered and documented.

Recommendation Box #3

How should sediments be spiked with a chemical or other test material?

- Regardless of the spiking technique used, care should be taken to ensure complete and homogenous mixing.
- Replicate subsamples should be analyzed to confirm homogeneous mixing.
- Moisture content should be determined on triplicates for each sample so that the spike concentration can be normalized on a dry weight basis.
- Wet spiking is recommended over dry spiking methods.
- Generally speaking, the jar rolling method is more suitable than hand mixing for spiking larger batches of sediment.
- To ensure chemical equilibrium between the sediment and pore water in toxicity testing, spike sediments should be stored for at least one month, unless other information is available for the spiking material and sediment type.
- Direct addition of organic solvent carriers should be avoided because they might alter sediment chemistry and affect contaminant bioavailability. Shell coating methods should be used instead as this eliminates many of the disadvantages of solvent carriers.

5.3.1 Preparation for Spiking

Debris and indigenous organisms should be removed from sediment samples as soon as possible after collection to reduce deterioration of sediment quality due to decomposition of organic debris and dying infauna. If sediments are to be stored prior to spiking, they should be kept in sealed containers at 4 °C.

Regardless of the spiking technique used, care should be taken to ***ensure complete and homogenous mixing*** (See Section 4.4). It is recommended that chemical analyses be conducted to verify that concentrations of the spiked contaminants are uniform throughout the mixed material. Three or more subsamples of the spiked sediment should be randomly collected to determine the concentration of

the substance being tested. In general, the coefficient of variation (CV) should be ≤20% for homogeneity of mixing to be considered sufficient (ASTM, 2000a; Northcott and Jones, 2000).

Temperatures should be kept cool during spiking preparation (e.g., 4° C) due to rapid physicochemical and microbiological alterations which might occur in the sediment that, in turn, might alter bioavailability and toxicity (ASTM, 2000a; Environment Canada, 1995). If spiking PAH compounds, it might be important to conduct spiking in the dark, or at least under low light as PAH toxicity has been shown to increase under ultraviolet light (Ankley et al., 1994).

It is recommended that a subsample of the spiked sediment be analyzed for at least the following parameters: moisture content, pH, ammonia, total organic carbon (TOC), acid volatile sulfide (AVS), particle size distribution, and background levels of the chemical(s) to be spiked. Further characterization may include analyses of total volatile residue, pore water salinity (before and after any sieving), chemical oxygen demand, sediment oxygen demand, oxidation-reduction potential (Eh), metals, total chlorinated organic content, chlorinated organic compounds, and polycyclic aromatic hydrocarbons (see Appendix G for more information on physicochemical parameters often measured on sediments). It is particularly important to determine the TOC concentration if the sediment is to be spiked with a nonionic organic compound, as organic carbon is the primary binding phase for such compounds (DiToro et al., 1990). Similarly, the concentration of AVS (the primary binding phase for cationic metals in anoxic sediments) and TOC should be measured after spiking with a cationic metal (Ankley et al., 1996; Leonard et al., 1999).

The sediment moisture content measurement is used to standardize the amount of chemical spiked on a dry weight basis (see Appendix G). Generally, the moisture content should be determined on triplicates for each sample by measuring the weight lost following 24 h of oven-drying at 105 °C. After drying, the samples should be cooled to room temperature in a desiccator before taking dry weight measurements (Yee et al., 1992). The mean wet density, expressed as mg water/cm^3, is measured by using the same drying method on known sediment volumes. This allows spiking to be normalized from a volume basis to an equivalent dry weight basis.

5.3.2 Methods for Spiking

Spiking of both wet and dry sediments is common, but wet spiking is recommended because drying might reduce the representativeness of the sample by changing its physicochemical characteristics (ASTM, 2000a). Methods differ mainly in the amount of water present in the mixture during spiking, the solvent used to apply the toxicant, and the method of mixing. Generally speaking, the jar rolling method is more suitable than hand mixing for spiking larger batches of sediment.

In addition to the above techniques, sediments may be spiked by hand stirring using a scoop or spatula, as long as the homogeneity of the mixture is verified. Eberbach and gyro-rotary shakers have also been used effectively to mix spiked sediments (Stemmer et al., 1990a). Less commonly, chemical(s) are added to the water overlying the sediment and allowed to sorb with no mixing (Stephenson and Kane, 1984; O'Neill et al., 1985; Crossland and Wolff, 1985; Pritchard et al., 1986).

Sediment Rolling

One of the recommended wet sediment rolling techniques requires a specific jar-rolling apparatus, first described by Ditsworth et al. (1990). Many other jar-rolling apparatuses are available, ranging in size and options available. This "rolling mill" method has been used to homogenize large volumes of sediments spiked with metals and non-ionic organic compounds. The primary disadvantage of this method is that the mixing apparatus must be constructed or purchased.

The jar-rolling apparatus used by Ditsworth et al. (1990) consists of eight parallel, horizontal rollers powered by an electric motor through a reduction gear, belts, and pulleys, which rotate cylindrical vessels containing the substrate mixtures. Mixing is accomplished gravimetrically by slowly rolling the jars (gallon-sized jars can be rolled at approximately 15 rpm). Optimally wetted, individual substrate particles adhere to each other and to the wall of the revolving jar until they cascade or tumble down the surface of the substrate mass. Dilution water may be added to the substrate before rolling to adjust the sediment-to-water ratio for optimal mixing. If oxidation is a concern (for example, if the sample will be analyzed for metals), jar contents might need to be maintained in an inert atmosphere. If PAHs are of concern then jars should be shielded from light (Ankley et al., 1994).

Considerations
When rolling sediment:

- Jars should not be overfilled, as this will reduce mixing efficiency.
- Prolonged rolling (e.g., > 1 wk) should be avoided to minimize physicochemical changes to the sediment.

Each jar should be loaded with the required amount of wet base sediment (with a calculated mass of dry sediment required for the test) prior to introduction of the toxicant. Several 1-cm diameter holes of different depths should be punched into the sediment to provide more surface area for the initial distribution of the test material. A predetermined volume of the stock solution or a serial dilution of the stock should be used to spike each jar load of sediment. A volumetric pipette should be used to distribute each aliquot onto the top surface and into the holes of the sediment in each jar. Sediments should be spiked sequentially, proceeding from low to high concentrations of test material, to minimize cross-contamination. Control substrates should be prepared by adding an equivalent volume of dilution water to a jar loaded with unspiked sediment. After spiking, all jars and their contents should be processed identically.

Typically, jars should be rolled for greater than two hours to achieve sample homogeneity. Jars should be closely monitored during the first hour of rolling to ensure proper mixing of substrates. After rolling for approximately 15 min, mixing efficiencies of the substrates can be judged visually. If a sediment displays excessive cohesiveness, as indicated by agglomerating or balling, the jars should be opened and an aliquot of appropriate dilution water (50 mL of either saltwater or freshwater depending on the source of the sediment) added to each substrate to increase the fluidity. This procedure should be repeated as necessary until the operator visually observes that all substrates are tumbling without forming balls. Adding water in small rather than large aliquots can prevent over-saturation of the sediment. Over-saturation is undesirable because excess water must be decanted following rolling, prior to sediment testing.

After rolling, the jars should be gently shaken to settle sediment that adhered to the walls. They may be set upright and stored overnight in the dark at room temperature or at an alternate temperature (e.g., 4° C) depending on the study objectives. After equilibration (see Section 5.3.3) and prior to distributing the sample to test chambers, additional rolling for two hours will help integrate interstitial water into the sediment.

Sediment Suspension Spiking

The sediment suspension technique (Cairns et al., 1984; Schuytema et al., 1984; Stemmer et al., 1990a; b; Landrum and Faust, 1991; Landrum et al., 1992) is the simplest of the three spiking techniques and requires the least equipment. The method involves placing dilution water and sediment together in a 1-L beaker. The desired amount of toxicant, dissolved in dilution water, is

added to the beaker. The mixture should be stirred at a moderate speed with a stir bar, or mechanical stirrer, for a minimum of four hours. The sediment in the beakers should then be allowed to settle and equilibrated at the appropriate test temperature as specified in the test method. The excess water overlying the sediment is decanted and discarded, and the sediment is distributed to the test containers (Environment Canada, 1995).

Slurry Spiking

The slurry technique (Birge et al., 1987; Francis et al., 1984; Landrum and Faust, 1991; Landrum et al., 1992) requires a minimum of equipment and involves less water than the sediment suspension technique. A 250-g dry weight sample of sediment is placed in a 500-mL Erlenmeyer flask. Via a 25-mL aliquot of distilled, deionized water, a sufficient concentration of the materials of interest is added to obtain the desired sediment concentration (mg/kg, dry weight basis). Control (unspiked) sediment receives a 25-mL aliquot of distilled, deionized water having no added materials. The sealed flask may be mixed using various methods such as continuous agitation in a shaker for five days (Birge et al., 1987) or vigorous shaking for 60 seconds, twice daily for seven days (Francis et al., 1984). Following mixing, the sediment suspensions should be centrifuged to remove water. The moisture content of the sediment should be approximately 15% to 20% after centrifugation. After removal of excess water, the prepared sediment can be placed in the exposure chambers and covered with dilution water according to the specific test methods. This procedure often yields sediment having its original moisture content.

5.3.3 Equilibration Times

Prior to distributing the spiked sediment to containers for toxicity testing or chemical analyses, ***the spiked sediments should be stored for a sufficient time to approach chemical equilibrium in the test material between the sediment and interstitial water.*** Equilibration times for spiked sediments vary widely among studies (Burton, 1991), depending on the spiking material and sediment type. For metals, equilibration time can be as short as 24 h (Jenne and Zachara, 1984; Nebecker *et al*., 1986), but one to two weeks is more typical (ASTM, 2000a). For organic compounds with low octanol-water partition coefficients (K_{ow}), equilibration times as short as 24 h have been used (Dewitt *et al*., 1989). Some organic contaminants might undergo rapid microbiological degradation depending on the microbial population present in the sample. In these cases, knowledge of microbial effects might be important in defining an appropriate equilibration period. Organic compounds with a high partition coefficient might require two months or more to establish equilibrium (Landrum et al., 1992). Boundaries for the sorption time can be estimated from the partition coefficient, using calculations described by Karickhoff and Morris (1985a, b). It is important to recognize that the quantity of spiked chemical might exceed the capacity of the test sediment system, prohibiting equilibrium.

For research purposes, unless definitive information is available regarding equilibration time for a given contaminant and sediment concentration, a one-month equilibration period is recommended, with consideration that two months might be needed in some instances (USEPA, 2000d). For regulatory programs, however, sample holding time should not exceed 2 weeks. Therefore, for these programs spiking equilibration time should not exceed 2 weeks. Periodic monitoring during the equilibration time is highly recommended to empirically establish stability of interstitial water concentrations (USEPA, 2000d). Sediment and interstitial water chemical concentrations should also be monitored during long-term bioassay tests to determine the actual chemical concentrations to which test organisms are exposed, and to verify that the concentrations remain stable over the duration of the test.

5.3.4 Use of Organic Solvents

Direct addition of organic solvents should be avoided if possible, because they might dramatically affect sediment geochemistry and alter bioavailability (USEPA, 2000d). However, many organic materials require use of a solvent to adequately mix with the sediment. If an organic solvent is to be used, the solvent should be at a concentration that does not affect test organisms and should be uniform across treatments. Further, both solvent control and negative control sediments should be included in tests with solvents. The solvent concentration in the control should equal the treatment concentration and should be from the same batch used to make the stock solution (ASTM, 2000a).

To reduce the possibility of solvent-related artifacts, the spiking process should include a step which allows the solvent to evaporate before addition of sediment and water followed by rolling (McLeese et al., 1980; Muir et al.,1982; Adams et al., 1985). Highly volatile organic compounds have been spiked into sediments using co-solvents followed by shaking in an aqueous slurry. When highly volatile compounds are used, immediate testing in covered flow-through systems is recommended (Knezovich and Harrison, 1988).

There is some uncertainty concerning artifacts introduced by the use of solvents. The use of a polar, water soluble carrier such as methanol was found to have little effect on the partitioning of nonpolar compounds to dissolved organic matter at concentrations up to 15% carrier by volume (Webster et al., 1990). However, another study showed that changes in partitioning by a factor of approximately two might occur with 10% methanol as a co-solvent for anthracene sorption (Nkedi-Kizza et al., 1985). The effect of carrier volume on partitioning of organic chemicals in sediments is equivocal. However, because solvents might be either directly or indirectly toxic to the test organisms, caution should be taken to minimize the amount of carrier used. In addition, the use of a carrier such as acetone might result in faster equilibration of spiked organic compounds (Schults et al., 1992).

Shell coating techniques which introduce dry chemical(s) to wet sediment have also been developed, principally to eliminate the potential disadvantages of solvent carriers. The chemical may be either coated on the inside walls of the container (Ditsworth et al., 1990; Burgess et al., 2000) or coated onto silica sand (Driscoll et al., 1997; Cole et al., 2000). In each shell coating method, the chemical is dissolved in solvent, placed in a glass spiking container (with or without sand), and the solvent is slowly evaporated prior to addition of the wet sediment. Wet sediment then sorbs the chemical from the dry surfaces. It is important that the solvent be allowed to evaporate prior to adding sediment or water.

5.4 Preparation of Sediment Dilutions

Spiked or field-contaminated sediments can be diluted with whole sediment to obtain different contaminant concentrations for concentration-effects testing. The diluent sediment should have physicochemical characteristics similar to the test sediment, including organic carbon content and particle size, but should not contain concentrations of contaminants above background levels (ASTM, 2000a; Burton, 1991). Diluent sediment has included formulated sediment as well as known reference site sediment. Diluted sediment samples should be homogenized and equilibrated in accordance with procedures described in Sections 4.4 and 5.3.3, respectively.

The diluent sediment should be combined with the test sediment in ratios determined on a dry weight basis to achieve the desired nominal dilution series (DeWitt, personal communication). Volume to volume dilutions have also been performed (e.g., Schlekat et al.,1995; Johns et al., 1985), but weight to weight dilutions are preferred because they provide more accurate control and enable a more straightforward calculation of dose-response curves.

Results from dilution experiments should be interpreted with care. There are often non-linear responses due to non-equilibrium, non-linear sorption-desorption processes that cannot always be adequately controlled (Nelson et al., 1993). Nelson et al. (1993) found that analyses of diluted sediments did not match nominal concentrations as estimated by physical characteristics. They suggested that chemical characterization is needed to determine effects of manipulations (i.e., mixing) and resulting changes (i.e., oxygenation of complexing agents such as acid volatile sulfides).

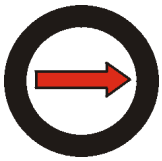
5.5 Preparation of Sediment Elutriates

Many studies of sediment toxicity have evaluated aqueous extractions of suspended sediment called elutriates. The elutriate method was initially developed to assess the effects of dredging operations on water quality (U.S. ACOE, 1976). Elutriate manipulations are also applicable to any situation where the resuspension of sediment-bound toxicants is of concern, such as bioturbation and storms, that might disturb sediments and affect water quality (USEPA/ACOE, 1991, 1998; Ankley et al., 1991). USEPA/ACOE (1998) lists eighteen freshwater and saltwater aquatic organisms as candidates for elutriate toxicity testing. Standard effluent toxicity test procedures are also appropriate for elutriates, including tests with various vascular and non-vascular plant species (Ingersoll, 1995).

Elutriate tests are not intended to reflect the toxicity of interstitial waters or whole sediments, as there are differences in contaminant bioavailability in the two types of media (Harkey et al., 1994). In general, elutriates have been found to be less toxic than bulk sediments or interstitial water fractions (Burgess et al., 1993; Ankley et al., 1991), although in some studies elutriates have been found to be more toxic (Hoke et al., 1990) or equally as toxic (Flegel et al., 1994) relative to interstitial water.

While there are several procedural variations, the basic method for elutriate preparation involves combining various mixtures of water and sediment (usually in the ratio of 4 parts water to 1 part sediment, by volume) and shaking, bubbling or stirring the mixture for 1 hour (Ross and Henebry, 1989; Daniels et al., 1989; Ankley et al., 1991; Burgess et al., 1993; USEPA/USACOE, 1991, 1998). It is likely that chemical concentrations will vary depending on the elutriate procedure used. Specific program guidance should be consulted as appropriate. The water phase is then separated from the sediment by settling and/or centrifugation (Note: the dredging remediation program does not always require centrifuging elutriates). Once an elutriate has been prepared, it should be analyzed or used in biological tests immediately, or as soon as possible thereafter. It should be stored at 4 °C for not longer than 24 h, unless the test method dictates otherwise (Environment Canada, 1994; USEPA/ACOE, 1991, 1998). For toxicity test exposures exceeding 24 h, fresh elutriate should be prepared daily.

Filtering the elutriate is generally discouraged, but it might be prescribed for some toxicity tests. Filtration can reduce the toxicity of sediment elutriates due to sorption of dissolved chemicals on the filtration membrane and retention of colloids. If colloidal material needs to be removed, serial or double centrifugation is generally a preferred alternative. If an elutriate must be filtered, it is recommended that only pre-treated filters be used and that the first 10 to 15 mL of the elutriate to pass through the filter be discarded (Environment Canada, 1994). Testing with a filtered elutriate should include an assessment to determine the extent of analyte adsorption/desorption to/from the filter.



Recommendation Box #4

How should sediment elutriates be performed?

- ➡ Combine 1 part sediment with 4 parts water unless a specific program stipulates otherwise.
- ➡ The sediment-water mixture should be vigorously shaken, bubbled or stirred for 1 hour.
- ➡ Centrifugation is a useful means for isolating the water phase (elutriate).
- ➡ Once prepared, the elutriate should be analyzed or tested as soon as possible.
- ➡ Store elutriate at 4° C with little or no headspace until analysis.
- ➡ Filtering the elutriate (to remove colloidal material) is generally not recommended. Use double (serial) centrifugation if appropriate.