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Sediment Spiking Methodologies for Nanomaterial Powders and Aqueous Suspensions for Aquatic Toxicity Bioassays

Scientific Operating Procedure Series: SOP-T-3

Jessica G. Coleman, Alan J. Kennedy, Jacob K. Stanley,
and Lauren Rabalais

June 2018

Environmental Laboratory

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Abstract

Homogenous dispersions of engineered nanomaterials (ENMs) for use in bioassay media for exposures/characterizations are crucial in ecotoxicology assays of nanomaterials in order to obtain reliable and repeatable results. Further, selection of the method for spiking sediment should consider the functional feeding behavior of benthic organisms which impact the way they are exposed to ENMs in the sediment; therefore, this procedure provides step-by-step methods for spiking nanoparticle working stock suspensions into or onto the surface of sediment for bioassays based on test organism functional group. Users are directed to follow the section relevant to their testing need and parent material (e.g., aqueous based-ENMs or powder ENMs). If followed correctly, near-homogenous particle dispersions of the reference materials and a best case scenario for dispersion of test materials in sediment/test water should be achieved using this procedural guidance.

Contents

Figures and Tables

Figures

Tables

Preface

This procedure was developed for the Headquarters, U.S. Army Corps of Engineers (USACE) under the Engineer Research Development Center (ERDC) Environmental Quality and Technology (EQT) Research Program titled "Environmental Consequences of Nanotechnologies." Procedures link to the ERDC NanoGRID (Guidance for Risk Informed Deployment) framework for testing the exposure and hazard of nanotechnology Environmental Health and Safety (EHS). The technical monitor was Dr. Elizabeth Ferguson.

The work was coordinated by the Environmental Risk Assessment Branch (EPR) of the Environmental Processes and Engineering (EPE) Division at the Engineer Research and Development Center - Environmental Laboratory (ERDC-EL). Dr. William Nelson was the Branch Chief, CEERD-EP-R, Mr. Warren Lorentz was the Division Chief, CEERD-EP-E; and Dr. Elizabeth Ferguson was the Technical Director for Environmental Quality and Installations. The Deputy Director of ERDC-EL was Dr. Jack Davis and the Director was Dr. Ilker Adiguzel.

COL Bryan S. Green was the Commander and the Director was Dr. David W. Pittman.

Acronyms and Abbreviations

ASTM	American Society of Testing Materials
DI	De-ionized
DLS	Dynamic Light Scattering
DoD	Department of Defense
EL	Environmental Laboratory
EHS	Environmental Health and Safety
ENM	Engineered Nanomaterials
EPE	Environmental Processes and Engineering
EPR	Environmental Risk Assessment Branch
EQT	Environmental Quality and Technology
ERDC	Engineer Research Development Center
FFF	Field Flow Fractionation
g	Gram
HQUSACE	Headquarters, U.S. Army Corps of Engineers
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Organization for Standardization
L	Liter
mL	Milliliter
NM	Nano Materials
NP	Nanoparticals
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
QA/QC	Quality Assurance/Quality Control
RM	Reference Material
RPM	Revolutions Per Minute

SERDP	Strategic Environmental Research and Development Program
SLM	Selective Laser Melting
SOP	Scientific Operating Procedure
TEM	Transmission Electron Microscope
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
USGS	United States Geological Survey
UV-Vis	Ultraviolet Visible Spectroscopy
Wt	Weight

1 Introduction

This Scientific Operating Procedure (SOP) was developed for creating near-homogenous dispersions of engineered nanomaterials (ENMs) into sediment for bioassay testing. The SOPs in this series will guide users in best practice material preparation techniques. This protocol was developed specifically using procedures created for metal ENMs, but may have broader applications. The SOPs serve as step-by-step guidance under the Engineer Research Development Center (ERDC) Environmental Consequences of Nanotechnologies. This SOP combines best laboratory practices available from the literature and professional experience of the ERDC and United States Geological Survey (USGS) research scientists.

1.1 Background

Sediment toxicity tests and bioaccumulation tests play an important role in the risk assessment process, especially for many ENMs that tend to agglomerate and settle from suspension in biologically relevant media. Thus, sediment is a likely repository and exposure vector for ENMs. While processes for spiking sediment with traditional contaminants exist (U.S. EPA 2001; ASTM 1706-05), researchers are still refining the methods for spiking and testing with ENMs. The issue is further complicated by lack of consensus between researchers concerning spiking methodologies that will result in the most homogenous distributions (OECD 2012), although standardized testing systems, vessels, and dispersion methods are required (Peterson et al. 2015). This document details a step-by-step procedure for spiking powder and aqueous ENMs into and onto sediment for toxicological bioassays. The methods are based on sediment testing protocols (U.S. EPA 2001; ASTM 2010), peer reviewed literature (Handy et al. 2012; Stanley et al. 2010; Coleman et al. 2013; OECD 2004; Mwangi 2010; Mwangi 2011; Brumbaugh et al.2013; Petersen et al. 2015), and best laboratory practices developed by ERDC and USGS scientists.

Contaminated sediment can pose risk to both benthic dwelling in-vertebrates and organisms higher up the trophic chain (SERDP 2008). Sediments also act as a repository for contaminants released into aquatic environments. Consequently, there have been multiple studies which show that ENMs settle and reside in/on the sediment of an aquatic environment (Handy et al. 2012; Coleman et al. 2013; Stanley et al. 2010; Kennedy et al.

2009, 2010). Benthic invertebrates have diverse phylogenies, anatomy (soft bodied, exoskeleton, shell), habits (infaunal, epibenthic) and feeding strategies (filter feeding, surface deposit feeding, subsurface deposit feeding). Based on feeding strategy and habitat preference, invertebrates may be exposed to contaminants in a variety of different ways such as through filter or deposit feeding, and burrowing (Coleman et al. 2014; NRC 2003). The way in which an organism feeds and resides in the sediment can greatly impact its risk at coming into contact with ENMs. Due to these differences, the present protocol is divided into two sections. The first section details direct ENM spiking into sediment for invertebrates which are infaunal/epibenthic and will likely be exposed through direct contact with sediment. The second section details procedures for indirect spiking into the water column for settling on sediment surface for epibenthic/water column dwelling organisms and suspension feeders. Users are directed to select the method which best fits their exposure need.

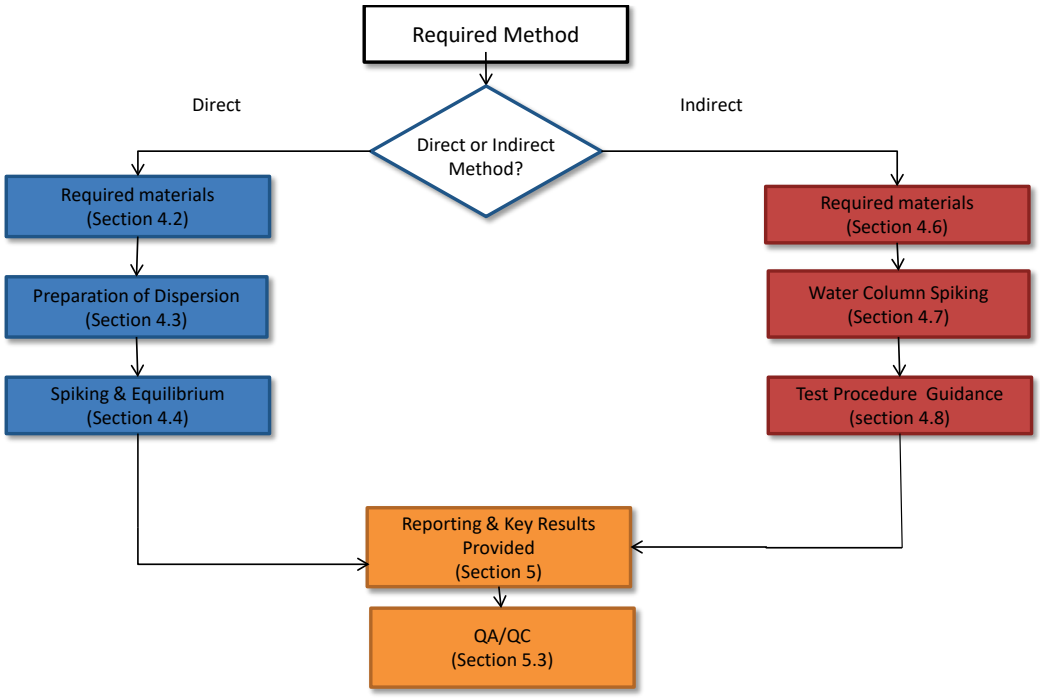
1.2 Scope

Two different sediment spiking methods are included in this SOP based on grouping the functional feeding and behavioral ecology of organisms commonly used for toxicity testing: (1) Direct sediment spiking method for subsurface sediment burrowing (infaunal) invertebrates and (2) Indirect spiking for water column/ sediment surface dwelling (epibenthic) invertebrates. The procedure differs in delivery methods for ENMs into sediment test based on user needs and test organism exposure routes in order to establish the worst case exposure and therefore, most conservative hazard value for the target organism. The two sections can be utilized separately or in tandem. Both methods may be used when it is not clear which will produce the most conservative hazard value. While the basic spiking methods may be applicable to a broad range of particles, carbon nanotubes are more difficult to homogenize and detect in sediment, resulting in some challenges to obtain optimal dispersions.

It is also recommended that a reference material be spiked into/onto a standardized artificial control sediment (OECD 2004) and conducted alongside the test material for validation of SOP procedures and consistency in cases where the artificial soil will support the test species of interest and duration of the exposure. Users should be aware that if the peat layer of the Organisation for Economic Cooperation and Development (OECD) sediment moves to the surface of the sediment, as observed by Mwangi (2010), the nanomaterials may move with it resulting in a less

homogenous exposure. The figures included in section 4 of this procedure includes a comparison between spiking dry nano powder (NIST SRM 1898 TiO2) into sediment versus utilizing the direct sediment spiking method recommended in this SOP.

Figure 1. Conceptual SOP flow chart.



2 Definitions

- Agglomerate - In nanotechnology, an assembly of particles held together by relatively weak forces (e.g., van der Waals or capillary), that may break apart into smaller particles upon processing.
- Dispersion Stability - Resisting change or variation in the initial properties of a dispersion over time (ISO 13097 2013).
- Working stock - Dispersion of ENM concentrate from manufacturer in aqueous suspension for bioassay media spiking/characterization.
- Aqueous media - Utilized in biological testing, bioassays, characterization, etc. Media may include, but is not limited to, moderately hard reconstituted water, hard reconstituted water, ultrapure water, de-ionized water, cell media, etc.
- Functional feeding group - Classification of organisms based on behavior-driven food acquisition.
- Infaunal Invertebrates - Live on sediment/substrate surface.
- Epibenthic Invertebrates - Live beneath sediment/substrate surface.

3 Procedures

3.1 Procedural overview

Procedures for creation of working stock from ENM aqueous suspensions or powders are provided in ERDC-SOP-T-1 (Coleman et al. 2014). Users are directed to utilize these procedures to create working stocks for ENM dispersions.

Guidelines are provided for direct and indirect (water column) sediment spiking. Field collected sediment can create a large amount of variability for ENM exposures due to its differing characteristics. Therefore, if testing a field collected sediment (characterized according to OECD 219 2004), additional testing of artificial sediment containing a known composition such as grain size and total organic carbon is recommended. Reference testing in the present SOP utilizes artificial control sediment formulated from OECD Guideline 218(2004).

Procedures for spiked sediment to reach equilibrium in section 3.4 are adapted from U.S. EPA 2001. Holding times after spiking sediment with a material vary widely (two days to two months). However, shorter periods may not allow sufficient equilibration or represent environmentally relevant (aged) ENM (and associated dissolved species, if applicable) bioavailability in sediment. Thus, an equilibrium period of one month after spiking is generally recommended (as described in U.S. EPA 2001 for general contaminants). In some cases, such as with carbon nanotubes, ENMs may not reach an equilibrium, but rather undergo physical mixing (Mwangi 2010). Users should be aware of this possibility when initiating an exposure with a non-metal ENM. Users are directed to consult section 5.3.3, EPA 2001 for more information.

3.2 Materials and methods: direct ENM sediment spiking

3.2.1 Materials

- Separatory funnel (size dependent on required volume of stock)
- Ring stand
- Plastic or glass container for sediment mixing; size is dependent on volume of sediment needed
- Mechanical table-top mixer and stand
- Plastic laboratory film or plastic container lid

- Metal weighing dish
- Roller mill
- 2–4 L glass or plastic jars with lid
- 1L glass beaker or similar based on test need

Specific materials may differ based on test organism and endpoint desired.

3.2.2 Sample preparation

3.2.2.1 *ENM working stock preparation for sediment spiking*

Chemical Analysis: Users should consult the manufacturer and literature to determine if their material has the potential to contain impurities. If impurities are suspected, two testing options are available: (1) the particles can be tested as-is to determine the worst case implications (though the impurity should be well characterized during the exposure); or (2) the ENM concentrate may be purified according to Miller and Chapell (2015) to attempt to assess only the implications of the nanoparticle itself. Next, particles should be characterized through Dynamic Light Scattering (DLS) and Selective Laser Melting (SEM) and/or Transmission Electron Microscope (TEM) according to Weiss and Moser (2015).

Working Stock Preparation: Powder and aqueous ENM working stocks should be prepared and characterized according to Coleman (2015). For accurate concentration, it is recommended to analyze the ENM working stock via ICP-MS prior to sediment spiking.

3.2.2.2 *Sediment preparation*

Reference sediment should be formulated according to OECD Guideline 218 (2004) to run in conjunction with test sediments. For test specific sediments, process and homogenize according to U.S. EPA (2001), specifically section 5.3. Briefly, a mechanical mixer is recommended for homogenization. Post mixing, no less than three sub-samples should be collected for background chemical analysis.

3.3 Analysis: direct ENM spiking into sediment

3.3.1 Sediment and working stock spiking calculations

Moisture content must be determined prior to spiking to standardize the chemical spiking on a dry weight basis. For further details, see section 5.3.1 in EPA 2001. To obtain the weight, utilize the formula below for n=3:

- Aluminum pan Wt (g)
- Pan + 1 g wet sediment
- Pan + 1g of sediment post 24-hour drying time in oven set to 105°C

Equation (1) wet/dry weight ratio:

$$b\text{-}a\ /c\text{-}a = wet/dry\ ratio \qquad \text{(Equation 1)}$$

Obtain wet/dry ratio average for n=3

Equation (2) wet weight (utilizing averaged values):

$$b\text{-}c/b = wet\ weight \qquad \text{(Equation 2)}$$

Extrapolate wet weight of sediment to volume of sediment and working stock needed for bioassay.

3.3.2 Working stock drip method

After preparing and characterizing the ENM stock according to Weiss and Moser (2015), calculated working stock volume should be placed in a separatory funnel held using a ring stand (Figure 2).

Position sediment container underneath funnel (Figure 2).

Secure table top mixer in stand, place impeller into center of sediment container with tip placed two-thirds to half way down into the total material volume depending on sediment type and moisture content. The position and volume should ensure a vortex and complete mixing of the sediment.

Slowly turn on the mixer to begin sediment mixing. The desired revolution per minute (RPM) of the mixer will be dependent on the volume and

viscosity of the sediment, although a vortex should be present in the sediment to ensure proper mixing.

Slowly drip the ENM working stock into the sediment at a drip rate of 5–20 drops per second. If necessary to maintain stability, an additional small mechanical mixer with prop may be used to keep the nanomaterials dispersed. Alternatively, particle dispersions can be maintained in a beaker while mixing (magnetic stir bar, aeration, mechanical mixing) and the suspension can be slowly delivered by pump. However, loss to the tubing must be first assessed.

Once all of the ENM working stock has been added to the sediment, cover the sediment container with plastic laboratory film or pre-fitted lid with an opening around the impeller. Allow sediment to mix in place for four hours. In addition, at 0 and 2 hours, a spatula should be used to manually mix the sediment and scrape the inside of the container to ensure complete mixing.

After four hours, turn off mixer and take three, 1 gram sediment samples from different locations in the container for chemical analysis.

3.4 Analysis: direct ENM spiking into sediment –obtaining equilibrium

Transfer sediment from mixing container directly over to a 2–4 L jar with lid.

Place tightly capped jar onto roller mill set to 20 RPM and allow aging for a one month period in a dark room, at a temperature range which is +/- 2 °C of the stated temperature range for the bioassay (Figure 3). Shorter (or longer) aging periods may be used if they can be scientifically justified to meet specific study objectives.

After one month (or alternatively justified aging period), remove sediment from roller mill and take four, 1 gram samples from different locations in the jar for chemical analysis.[1]

3.5 Analysis: direct ENM spiking into sediment - sediment toxicity testing

Test duration, parameters, organism, and chamber size will differ based on the specific bioassay method employed. The steps below provide a general guideline which can be modified as needed for test specifics.

Post ageing, sediment should be thoroughly re-homogenized mechanically and manually as described above in section 4.2.2.2, then evenly divided between test containers.

Overlying test water should be slowly added to sediment, through a turbulence reducing apparatus such as a small plastic lid or scoop.

If aeration is needed, allow water to settle within beakers for a minimum of 24 hours before adding air. If no aeration is needed, allow beakers to equilibrate 24 hours prior to organism addition.

Post equilibration, sample 1–5 mL of overlying water (1 cm above the sediment surface) for ICP-MS.

Test duration will vary based on the individual bioassay method selected.

Overlying water samples (1–5 mL) for ICP-MS should be taken at least once a week until test is completed.

At test termination, take a 1 mL overlying water (n=3) 1 cm above the sediment surface, then carefully remove remaining overlying water.

[1] Note: equilibrium time may vary based on material and sediment (see section 4.1). A one month minimum period is recommended to increase likelihood of equilibration of steady state, represent the fully transformed, and environmentally relevant bioavailability of the test material since most sediment exposures consider longer term impacts. Equilibrium may be particle dependent, undergoing physical mixing rather than true equilibrium (Mwangi 2010), or require extended aging based on the material (Brumbaugh et al. 2013). Users should be aware of this possibility when preparing their test media. At minimum, chemical analysis of sediment post-test termination must include ICP-MS of total concentration for metal nanoparticles (NPs).

Re-homogenize sediment from three replicates utilizing a table top mixer, then sample 1–3 g per replicate from four different locations in the sediment for a total of four samples per container of homogenized sediment. At minimum, chemical analysis of sediment post-test termination must include ICP-MS of total concentration for metal ENMs.

3.6 Materials: indirect (water column) ENM spiking

Materials

- Gloves
- Pipettes
- Metal spatula
- ENMs
- Balance

3.7 Analysis: indirect ENM water column spiking

Sediment volume, addition, and water volume should be established based on standardized toxicity methods for organism of choice. The reference test below generally follows the testing guideline in ASTM E 1706-05 (2010).

Once water and sediment volumes are calculated, and the sediment added to the beakers, users will refer to section 5.2.2.1 of ASTM E 1706-05 (2010) for material characterization and dispersion procedures.

The ENM stock dispersion may be used directly, or diluted into the volume of overlying water needed for each treatment concentration. Test concentrations are user defined but may be based on aquatic toxicity values of ENMs if known. The amount of ENM added will be based on the desired total treatment concentration in the sediment on dry weight basis. In the absence of toxicity information, a range finder study may be needed.

Spiked, overlying test water or the stock dispersion should be slowly added to sediment through a turbulence reducing apparatus such as a small plastic lid or scoop. Beakers should be allowed to equilibrate for a minimum of 24 hours. Post settling; collect a 1.5 mL water sample from one replicate per treatment for DLS and 1 mL for ICP-MS.

If aeration is needed, light bubbling is recommended (<100 bubbles per minute).

Add test organisms under the water's surface as detailed in the protocol relative to test organism, taking care not to disturb the bottom ENM layer.

Collect 1–5 mL overlying water samples at test initiation and termination (n=3) for measurement of dissolution on an ICP-MS.

Duration of test will be organism specific; the reference test is conducted for 10 days, static with no water renewals.

3.8 Analysis: indirect ENM water column spiking-test procedure and characterization guidance

When working with new or uncharacterized materials, it is recommended that settling rates of ENM powder are established prior to organism addition. Assessment of settling rates will be material dependent, but could include UV-Vis, turbidity, DLS, or visual observations.[1]

Acute, static tests are generally recommended over chronic exposures which require water changes. This recommendation is based on the theory that water changes can result in ENM loss and changes in the system.

In tests that require water changes to maintain water quality (e.g., U.S. EPA 2000), care must be taken not to disturb the bottom ENM layer. Water additions must be added slowly in an effort not to re-suspend ENMs.

At minimum, the removed overlying water (at each exchange, if applicable) and sediment samples are to be analyzed according to section 5.5.1.8 of ASTM E 1706-05 (2010) to gain an understanding if resuspended ENMs or dissolved metal species are being lost from the test system in order to determine if the most conservative hazard result was obtained.

If it is determined a substantial loss of test substance was lost from the system, one of the two following options should be executed:

[1] Martin, David P., Aimee R. Poda, Anthony J Bednar. In press. Quantifying nanoparticle release from nanotechnology: Scientific Operating Procedure Series*:* SOP-C-3. Environmental Lab. Vicksburg, MS: Engineer Research and Development Center.

- If possible to maintain organism health, the test may be repeated without water exchanges.
- The loss of material should be fully characterized and reported and a mass balance approach can be used to determine the mass of material still in the test system between each water exchange. This information can be used to calculate toxicology endpoints using a time-weighted average approach, as described in Petersen et al. 2015.

4 Reporting

4.1 Analysis of results: direct ENM spiking into sediment reference materials

4.1.1 Key results provided

The present protocol provides methods for spiking an ENM working stock into sediment for an aquatic bioaccumulation or toxicity bioassay. If followed correctly, the user should be able to create a near homogenous dispersion of ENMs in a sediment matrix at the start of the exposure based on best methods currently available. Results in Table 1 show little difference in average TiO2 concentrations in sediment spiked utilizing the working stock drip method.

Table 1. Measured TiO_2 concentrations post sediment spiking and ageing on roller mill through working stock drip method.

	Replicate sub-samples	concentration mg/kg	Average concentration mg/kg	Standard Deviation
Container A	1	5230	5217	32
	2	5230		
	3	5170		
	4	5240		
Container B	1	5180	5445	192
	2	5630		
	3	5530		
	4	5440		
Container C	1	5240	5132	273
	2	5150		
	3	5390		
	4	4750		

4.1.2 Potential test organisms and exposure methods

Table 2 provides a list of potential test organisms and exposure methods.

Table 2. Potential test organisms and exposure methods.

Functional Group	Organism Example		ENM Application
	Freshwater	Marine	
Infaunal	Chironomus dilutes Tubifex tubifex Lumbriculus variegatus	Leptocheirus plumulosus Eohaustorius estuaries Ampelisca abdita Nereis arenaceodentata Macoma nasuta	Sediment Spiking
Epibenthic	Hyalella Azteca Daphnia magna* Daphnia pulex*	Americamysis bahia Macoma nasuta (surface deposit feeder)	Sediment Spiking or Surface Application
Suspension Feeders	Corbicula fluminea	Oysters mussels clams	Surface Application

* Represents zooplankton which dwell in water column.

4.2 Quality assurance/quality control (QA/QC) considerations

During preparation, multiple factors can affect results. Characteristics which can affect dispersions include, but are not limited to, sample volume, sediment characteristics, pH, and particle characteristics (i.e., coatings, size, solution, etc). To enhance the potential of obtaining consistent results, it is suggested to compare RM in artificial sediment to ensure procedures are correctly and consistently applied during the sediment preparation. Furthermore, users should be aware that non-metal particles (i.e., carbon nano tubes) may behave differently in sediment exposures than metal ENMs which the present procedure is based on. Therefore, careful attention must be paid to indications of particles separating from sediment, resulting in a physical mixing rather than equilibrium (Mwangi 2010). Figures 2 and 3 show the set-up for direct ENM sediment spiking working stock drip method and the roller mill mixing sediment.

Figure 2. Set-up for direct ENM sediment spiking working stock drip method.

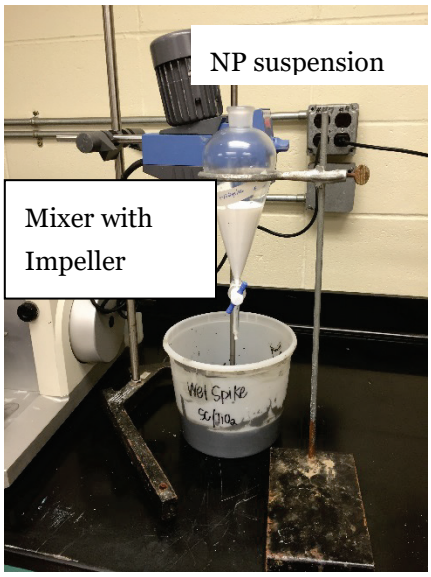


Figure 3. Roller mill mixing sediment.

References

American Society of Testing Materials (ASTM). 2010. Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates. E1706-05. West Conshohocken, PA: ASTM International.

Brumbaugh, W. G., J. M. Besser, C. G. Ingersoll, T. W. May, C. D. Ivey, C. E. Schlekat, and E. R. Garman. 2013. Preparation and characterization of nickel-spiked freshwater sediments for toxicity tests: Toward more environmentally realistic nickel partitioning. *Environmental Toxicology and Chemistry* 32(11):2482–2494.

Coleman, J. G., A. J. Kennedy, A. J. Bednar, J. F. Ranville, J. G. Laird, A. R. Harmon, C. A. Hayes, E. P. Gray, C. P. Higgins, G. Lotufo, and J. A. Stevens. 2013. Comparing the effects of nanosilver size and coating variations on bioavailability, internalization, and elimination, using Lumbriculus variegatus. *Environmental Toxicology and Chemistry 32*(9):2069–2077.

Coleman, Jessica G., Alan J. Kennedy, and Ashley R. Harmon. 2015. *Environmental Consequences of Nanotechnologies: Nanoparticle Dispersion in Aqueous Media: SOP-T-1*. No. ERDC/EL SR-15-2. Vicksburg, Ms: U. S. Army Engineer Research and Development Center.

Coleman, J. G., G. R. Lotufo, A. J. Kennedy, A. R. Poda, T. S. Rushing, C. E. Ruiz, and T. S. Bridges. 2014. Testing of various membranes for use in a novel sediment porewater isolation chamber for infaunal invertebrate exposure to PCBs. *Chemosphere* 106:65–69.

Handy, R. D., G. Cornelis, T. Fernandes, O. Tsyusko, A. Decho, T. Sabo-Attwood, and C. Metcalfe .2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. *Environmental Toxicology and Chemistry* 31(1):15–31.

International Organization for Standardization (ISO). 2013. Guidelines for the characterization of dispersion stability. TR 13097. Vernier, Geneva: Switzerland.

Kennedy, A. J., J. C. Gunter, M. A. Chappell, J. D. Goss, M. S. Hull, R. A. and Kirgan, J. A. Steevens. 2009. Influence of nanotube preparation in aquatic bioassays. *Environmental Toxicology and Chemistry* 28(9):1930–1938.

Kennedy, A. J., M. Hull, J. Bednar, J. Goss, J. Gunter, J. Bouldin, P. Vikesland, and J. Steevens. 2010. Fractionating nanosilver: Importance for determining toxicity to aquatic test organisms. *Environmental Science and Technology* 44(24):9571–9577.

Miller, L. F., and M. A. Chappell. 2015. *Purification and concentration of nanoparticles using diafiltration: Scientific Operating Procedure Series: SOP-P-1*. ERDC/EL SR-15-4. Vicksburg, MS: U. S. Army Engineer Research and Development Center.

Mwangi, J. N., 2010. Aquatic toxicity of one dimensional carbon nanomaterials. PhD diss., University of Missouri-Columbia.

Mwangi, J. N., N. Wang, A. Ritts, J. L. Kunz, C. G. Ingersoll, H. Li, and B. Deng. 2011. Toxicity of silicon carbide nanowires to sediment-dwelling invertebrates in water or sediment exposures. *Environmental Toxicology and Chemistry* 30(4):981–987.

Mwangi, J. N., N. Wang, C. G. Ingersoll. D. K. Hardesty, E. L. Brunson, H. Li, and B. Deng. 2012. Toxicity of carbon nanotubes to freshwater aquatic invertebrates. *Environmental Toxicology and Chemistry* 31(8):1823–1830.

National Research Council (NRC). 2003. *Bioavailability of contaminants in soils and sediments*. Washington, DC: The National Academies Press.

Organisation for Economic Co-operation and Development (OECD). 2004. *Guideline for the testing of chemicals No. 218: Sediment-water chironomid toxicity test using spiked sediment*. doi:. 10.1787/9789264070264-en.

Organisation for Economic Co-operation and Development (OECD). 2012. *Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials*. OECD Environment. Health and Safety Publications Series on the Safety of Manufactured Nanomaterials. No. 36. ENV/JM/MONO 40, Paris, France.

Petersen, E. J., S. A. Diamond, A. J. Kennedy, G. G. Goss, K. Ho, J. Lead, S. K. Hanna, N. B. Hartman, K. Hund-Rinke, B. Mader, N. Manier, P. Pandard, E. R. Salinas, and P. Sayre. 2015. Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. *Environmental Science and Technology* 49(16):9532–9547.

Stanley, J. K., J. G. Coleman, C. A. Weiss, and J. A. Steevens. 2010. Sediment toxicity and bioaccumulation of nano and micron-sized aluminum oxide. *Environmental Toxicology and Chemistry 29*(2):422–429.

Strategic Environmental Research and Development Program (SERDP). 2008. SERDP and ESTCP expert panel workshop on research and development needs for understanding and assessing the bioavailability of contaminants in soils and sediments. Workshop held on August 20–21, 2008, in Annapolis, MD. http://docs.serdp-estcp.org/index.cfm.

U. S. Environmental Protection Agency (U.S. EPA). 2001. *Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual*. EPA-823-B-01-002. Washington, DC: Office of Science and Technology.

U.S. Environmental Protection Agency (U.S. EPA), 2000. *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates*. EPA/600/R-99/064. Washington, DC: U.S. Environmental Protection Agency,

Weiss Jr., C. A., and R. D. Moser. 2015. *Sample preparation of nano-sized inorganic materials for scanning electron microscopy or transmission electron microscopy: Scientific Operating Procedure: SOP-P-2*. ERDC/GSL-SR-15-1. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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14. ABSTRACT

Homogenous dispersions of engineered nanomaterials (ENMs) for use in bioassay media for exposures/characterizations are crucial in ecotoxicology assays of nanomaterials in order to obtain reliable and repeatable results. Further, selection of the method for spiking sediment should consider the functional feeding behavior of benthic organisms which impact the way they are exposed to ENMs in the sediment; therefore, this procedure provides step-by-step methods for spiking nanoparticle working stock suspensions into or onto the surface of sediment for bioassays based on test organism functional group. Users are directed to follow the section relevant to their testing need and parent material (e.g., aqueous based-ENMs or powder ENMs). If followed correctly, near-homogenous particle dispersions of the reference materials and a best case scenario for dispersion of test materials in sediment/test water should be achieved using this procedural guidance.

15. SUBJECT TERMS

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Nanostructured materials
Environmental Toxicology--Testing
Biological assay

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