Fate of Nanoparticles in the Environment

Level 4 Quality Assurance Project Plan
(3/4/13, revised 2/23/16)

Exposure Methods and Measurement Division
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Athens, GA

Principal Investigators: Dermont Bouchard, Richard Zepp, Matt Henderson, Chris Knightes

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1 Project Management

1.1 Distribution List and Meta Data

Dr. Dermont Bouchard	Principal Investigator
Tammy Jones-Lepp	ECB Branch Chief
Margie Vazquez	EMMD Quality Assurance Officer

Project 18.02 Meta Data

1) RAP Number – CSS 18.02
2) Project Name: Emerging Materials-Engineered Nanomaterials
3) Project Lead: Kim Rogers
4) Project Lead Organization: NERL
5) Task Name: Currently in project planning, going from 4 tasks to three tasks
    a. Task 1: Nanoinformatics and Nanomaterial Database (Proposed Task Leads: Will Boyes and Souhail Al-Abed
    b. Task 2: Decision Tree and Case Studies (Proposed Task Lead: Kim Rogers. Proposed product POCs for MWCNT and graphene family materials (GFM) study: Matt Henderson and Dermont Bouchard)
    c. Task 3: Functional assays and predictive modeling (Lead: Paul Harten and Mike Hughes)
6) Task Lead: see above, EMMD mainly involved in tasks 2 and 3
7) Task Lead Organization:
8) Other Organization/POCs: see above
9) Internal POCs: Dermont Bouchard, Richard Zepp, Matt Henderson, Chris Knightes
10) Contractor/POC:
    a. Xiaojun Chang, NRC (working with Dermont Bouchard)
    b. Sharon Martin, SSA (working with Matt Henderson)
    c. Brian Avant SSA (working with Chris Knightes)
    d. Brad Acrey SSA (working with Richard Zepp)
11) Task Documents: In QATS, Task Plan entitled Transport and Fate of Nanomaterials in the Environment Task (Version Date: 6/26/13, start/end date: 10/1/13-9/30/17). This task plan will be replace within the next month with a new CSS Emerging Materials project plan. Dermont offered to forward along a copy.
12) QA Category: Level IV
13) QATS: JKIS-7BKR4C
14) QMP: None
15) QAPPs: Transport of Nanoparticles in the Environment (Last Revision 2/2/13) -> Modeling QAPP to be prepared.
16) SOPs: RSB-011.1 and RSB-012.1 -> SOPs were recently revised and will be routed through Rajbir for updating in the QA database

17) Audits (Including Data, Document, & Test Reviews):
    a. FY14: LCA conducted 8/19/14 -8/20/14 (Dermont Interviewed)
18) Field Plan: N/A
19) Records Management Plan:
20) Products.
    a. FY15 APR: Library of well-characterized nanomaterials for cross laboratory collaborative studies, materials are selected to test the role of physical/chemical parameters in determining nanomaterial activity in environmental and biological systems.
    b. FY18: Development of reference systems for functional assays that provide parameter estimates and process relationships for models of light-induced transformations of ENMs in aquatic systems
    c. FY18: Modeling Tools for Simulating Biomarker Response to ENM Exposures in Aquatic Systems
    d.

1.2 Task Organization

Independent research will be conducted by all Investigators on this task. This may include laboratory experiments, limited field investigations, data analysis, chemical modeling, preparation of publications, and oral presentations. All Investigators will assume responsibility for verifying results from the instruments that they (or their technicians, SEE employees, NRC associates, etc.) operate and maintain.

1.3 Problem Definition/Background

The unique electronic, mechanical, and structural properties of engineered nanomaterials (ENMs), their potential in drug delivery and other biomedical applications, as well as utilization in polymer composites, has led to increasing production of these versatile materials. There are currently more than 1600 consumer products reported to contain nanoscale materials; and the use of carbon-based nanomaterials (fullerenes, carbon nanotubes (CNTs), and graphene family materials) in these products trails only nano-scale silver and titanium. Carbon-based nanomaterials are also referenced in 40% of the nanotechnology patent applications submitted to the US Patent and Trademark Office from 1/2010–3/2011,[1] and upper estimates for US CNTs production are in excess of 1000 tons per year.[2] Clearly, as more carbon-based ENMs are produced and utilized in commerce, the potential for either permitted or accidental releases increases; and, concomitantly, the potential for human and ecological exposures also increases. These potential exposures underscore the importance of developing a clearer understanding of ENM transport and transformation in the environment.

In addition, here is a growing consensus that using the basic intrinsic properties of ENMs to parameterize environmental fate models is problematic due the resources required to determine these properties as well as the fact that important ENM properties are not

constant but a function of the media in which they are measured. Functional assays, which measure intermediate values that integrate key ENM characteristics in well-defined reference systems, will be used to generate input parameters for WASP8 aquatic fate modeling. Functional assays will be developed that describe ENM attachment to surfaces, phototransformations, and biomarker response in well-defined environmentally relevant reference systems.

1.4 Task Description

The general approach for using functional assays in ENM transport and fate modeling is depicted in Figure 1. In this conceptualization functional assays are developed for three key processes determining ENM environmental aquatic fate: ENM attachment to environmental surfaces (ex: ENM attachment to sediments), transformation processes (focus is on phototransformation or carbon-based ENMs), and biomarker response to ENMs with emphasis on adhesion to and interaction with biological macromolecules and/or aquatic organisms. While values for ENM-ENM aggregation are common in the literature, values for the heteroaggregation of ENMs with naturally occurring particulate matter, such as sediments and particulate organic matter are quite rare. For attachment to environmental surfaces, heteroaggregation in ENP-surface systems may be described by the heteroaggregation attachment efficiency parameter (α_{hett}) which represents the fraction of ENP-surface collisions that result in attachment. Transformations of ENMs by light-induced and other pathways potentially can affect exposure concentrations of ENMs and these processes can be addressed in process-based, differential mass balance models such as WASP. Here we examine transformations of pristine ENMs suspended in water and also transformations of ENMs incorporated into consumer products such as polymers with emphasis on carbon nanomaterials such as nanotubes and graphene-like substances. Photo-transformations can affect exposure concentrations of ENMs by conversions to breakdown products such as carbon dioxide, low-molecular weight products and metal ions. In addition, photo-transformations affect exposure, bioavailability and possibly toxicity by altering transport of the ENM via changes functional groups such as carboxyl groups that participate in hetero aggregation and other transport processes. Light-induced transformations of ENMs in aquatic environments can occur by two general pathways: direct photolysis by light absorbed by the ENM or indirect photoreactions that are initiated by natural substances such as NOM, metals and peroxides. To develop predictive relationships for modeling photo-transformations in surface waters we will develop reference systems for functional assays that include known irradiations in water containing constituents of natural waters that alter photoreactions by changing key parameters such as apparent quantum yields, light attenuation and reactive oxygen species concentrations. Data derived from biomarker functional assays describing the deposition of ENMs on biological substrates will be integrated with modeled ENM concentrations to develop ENM-biomarker response relationships.

The Water quality Analysis Simulation Program (WASP8) is a flexible, dynamic, mass-balance framework for simulating the fate and transport of environmental contaminants

in surface water systems. The objective of this work is to develop an enhanced version of WASP that incorporates particle attachment kinetics, phototransformations, and biomarker response to simulate ENM transport in surface waters using laboratory and field measured input data. To simulate ENM transport in aquatic systems, heteroaggregation values (α_{hett}) for ENM-sediment systems will measured under a range of reference background solution conditions to generate a matrix of α_{hett} input values for WASP8. Models for evaluating direct and indirect photoreactions of ENMs will also be developed and evaluated for inclusion in WASP8. Both direct and indirect pathways are affected by the composition of the medium surrounding the ENM and its photoproducts. Data and relationships for ENMs in consumer products and ENM/polymer composites will focus on release of the ENMs in weathering systems that can modulate key parameters such as light intensity, temperature and simulated rainfall. Kinetic relationships will be developed for predictive models that link release rates of the ENMs to these parameters. Interactions with biological organisms will be estimated using model membrane-quartz microbalance techniques for quantitating ENM deposition and uptake utilizing supported lipid bilayers and supported vesicle layers.

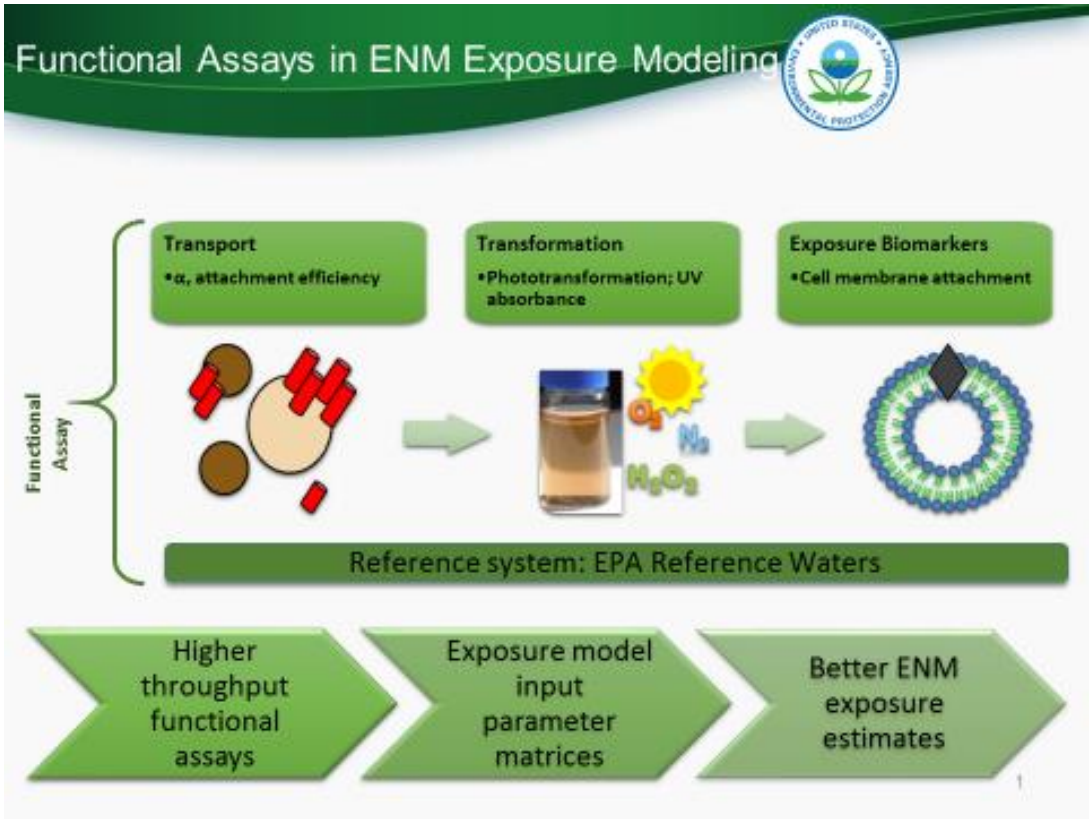

Figure 1. Role of functional assays in ENM exposure research.

(1) Leitch, M.E.; Casman, E.; Lowry, G.V., Nanotechnology patenting trends through an environmental lens: analysis of materials and applications. *J Nanopart. Res.* **2012**, *14*, 1283-1306.

(2) Hendren, C. O.; Xavier Mesnard, X.; Droge, J.; Wiesner, M.R., Estimating production data for five engineered nanomaterials as a basis for exposure assessment. *Environ. Sci. Technol.* **2011**, *45* (7), 2562−2569.

1.5 Quality Objectives and Criteria for Measurement Data

Proven peer-reviewed methods will be used when available. New measurement methods will be developed and tested as needed. HPLC-MS, GC-MS, LSC, UV/VIS, AFM, and DLS methods will be the main techniques used to characterize ENMs. For all quantitative measurements involving reactions, certain standard data quality indicators will be determined and evaluated for acceptability. These include:

a) precision - Acceptable precision for analysis of replicates of the same sample (i.e., same time point in a given reaction) will be in the range of 0 - 15% coefficient of variation (CV). (We define CV as the sample standard deviation divided by the mean and multiplied by 100.) Generally, duplicates or triplicates of a given sample will be analyzed. Acceptable precession for rate constants determined from multiple runs will be in the range of 0 - 30% CV.

b) accuracy - Generally, the method of spike-and-recovery will be used at least once to determine accuracy for each analytical technique that is used to quantify a given analyte. A representative solution-phase sample already containing a quantified amount of the analyte will be selected for spike-and-recovery. This sample will then be spiked with a known amount of additional analyte (using a high-concentration standard) and the final concentration of the analyte in the sample will be measured. The % recovery of the analyte (measured final concentration divided by target final concentration multiplied by 100) must be in the range of 75 - 125 % for acceptability. Note that the target final concentration after spiking should be between 1.5 and 2 times the initial measured concentration. Also, note that the volume of the spike should be less than 2% of the volume of the sample.

c) sensitivity - It is not possible to determine detection limits in advance since many different analytes in different matrices and many different analytical techniques will be used. The whole-method limit of detection (LOD) may be defined as the sample concentration that gives a signal-to-noise ratio (S/N) of greater than 3:1; the whole-method limit of quantification (LOQ) may be defined as the sample concentration that

gives a S/N of greater than 10:1 [J.M. Green, Anal. Chem. 68 (1996) 305A]. For most peer-reviewed publications the LOD may be discussed but most data values should ≥ the LOQ. Generally, for spectroscopic and chromatographic techniques, the detection limit will be set to the calibration sample concentration where the analyte signal is equal to 3 times the noise level (i.e., analyte peak height = 3 times the peak-to-peak noise in the baseline near the analyte peak.) No measurements will be acceptable below the detection limit. Once a detection limit has been established for a given analytical technique for a given chemical, data will no longer be acceptable if the detection limit is found, by routine checks (generally conducted weekly), to be greater than 3 times the established level. If the detection limit becomes unacceptable, corrective action (e.g., servicing the instrument) will be taken. The detection limit must return to an acceptable level (< 3 X the established limit) before further data is collected.

1.6 Special Training Requirements/Certification

No special training/certification is required of the personnel

1.7 Documentation and Records

For all laboratory generated data, all pertinent procedures, raw data, calculations, etc., are maintained in the laboratory notebook according to LOP 5400-3. Because computers are used in all phases of this research, cross referencing of computer files and experimental conditions are crucial, as is a data file backup procedure. All data collected by computer will be given a unique name with the date encoded therein. A log book will contain a list of all file names and detailed experimental conditions. Details are maintained and cross-referenced with computer filenames within the laboratory notebook. Generally, data stored in digital format will be backed-up over the network. Network drives are backed up daily by the contractor. A record of data backups is maintained in log books. Also, a current backup of the entire drive that contains system files is maintained.

2 Measurement/Data Acquisition

2.1 Sampling Process Design (Experimental Design)

Most of the sampling in this project will be batch sampling removing a liquid aliquot of a larger volume for instrumental analysis. Sampling methodologies are described in attached protocols and SOPs.

2.2 Sampling Methods

See protocols and SOPs in section 5.0.

2.3 Sample Handling and Custody

Stock solutions will be retained in glass or plastic containers and labeled with the date, the name of the preparer, and the concentration of solution. For discrete sampling of individual experiments, a sequential sample number will be used in conjunction with designated experiment identification number and experimental conditions. The record of all samples taken for each experiment will be in the technician's laboratory notebook as well as the cross-referenced computer data file.

2.4 Analytical Methods

Major instrumentation required is HPLC with diode array detector, Liquid Scintillation Counter, flow field flow fractionator, quartz crystal microbalance, atomic force microscope, and a photon correlation spectrometer. If it is not feasible for in-house repair or maintenance, a purchase order for 'one time' service is sought. In addition to these instruments, state-of-the-art personal computers are required (and currently available) for data acquisition and interpretation and for model development and testing.

2.5 Quality Control

This task will comply with Athens Laboratory Operating Procedure No. 5400-6, Performance and System Audit Procedures.

2.6 Instrument/Equipment Testing, Inspection, and Maintenance

Routine quality assurance functions will be carried out to insure that all the analytical equipment used in this research is functioning properly. Periodically throughout sample runs or randomly after machine calibration, sample blanks and quality assurance samples will be run in order to verify precision and accuracy of the instrument. If the instrument does not respond satisfactorily, then maintenance above the normal routine scheduled maintenance will be performed to correct for the problem. If the problem is beyond in-house repair, then a purchase order for 'one time' service is sought.

We will not describe routine quality assurance functions in this document for all the many analytical instruments that are used in this task because the details, which are based on instrument-specific “standard practices”, are quite different for each instrument. However, for illustration, our quality assurance functions for the HPLC diode array detector will involve monthly testing of lamp output to determine that its output is within product specifications.

2.7 Instrument Calibration and Frequency

All the analytical equipment used in this study will be calibrated prior to sample analysis. Also, quality assurance standards will be run to verify instrument response throughout and after each sample run. The details of calibration will differ for each instrument.

2.8 Inspection/Acceptance of Supplies and Consumables

All inspection of supplies and consumables will be performed by each investigator. Acceptance criteria will be based on if any of the supplies and consumables do not give the expected peak performances of a known standard or if the product does not meet the manufacturer's stated product specifications.

2.9 Data Acquisition Requirements (Non-direct Measurements)

Computer data bases and programs used will include Microsoft Excel and SigmaPlot softwares. Acceptance criteria will be based on manufacturer's stated product specifications.

2.10 Data Management

For all laboratory generated data, all pertinent procedures, raw data, calculations, etc., are maintained in the laboratory notebook according to LOP 5400-3. Because computers are used in all phases of this task, cross referencing of computer files and experimental conditions are crucial, as is a data file backup procedure. All data collected by computer will be given a unique name with the date encoded therein. A log book will contain a list of all file names detail experimental conditions. Details are maintained and cross-referenced by computer filename within the laboratory notebook. Data stored in digital format will be backed-up over the network. Network drives are backed up daily by the contractor. A record of data backups is maintained in log books. Also a current backup of the entire drive containing system files is maintained.

3 Assessment/Oversight

3.1 Assessments and Response Actions

Quality assurance audits are initiated by Division management and Laboratory QA staff. Based upon these audits, or as judged necessary by the PI, corrective action will be undertaken. Unresolved issues may be referred to the Branch Chief or the Division's QA officer.

3.2 Reports to Management

Informal progress reports will be made frequently to the Branch Chief, and where applicable, to the task manager. Journal articles, oral presentations, and reports are the primary format for reporting results of this task to the scientific community. All Division managers in the chain-of-command will review and clear these products.

4 Data Validation and Usability

4.1 Data Review, Validation, and Verification Requirements

This has been covered in the section 1.5 (Quality Objectives and Criteria for Measurement Data).

4.2 Validation and Verification Methods

Virtually all outputs from this task will be peer reviewed journal articles. Success can be judged through acceptance of articles in respected journals. Also, reviewer comments provide insight into success of the project.

In addition, at the discretion of the Investigators, further data quality evaluations will be undertaken if deemed prudent. This could include analysis of standards and matrix spikes that would be "blind samples" to the task personnel making quantitative measurements and reporting results. Also, blind or double-blind round robin studies may be undertaken.

4.3 Reconciliation with User Requirements

While the issue that this project addresses is of interest to all EPA Program Offices, they will not use quantitative results from this task directly in regulatory action. Rather the incorporation of transport process findings into site scale models will be the likely technology transfer mode.

5.0 Standard Operating Procedures and Protocols

Protocol to Determine Particle Size and Zeta Potential using the Malvern Zetasizer

SOP# RSB-011.1 effective 4/4/2014, revised 6/11/15

Exposure Methods and Measurement Division
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Athens, GA

Concurrences:

_____Date_____
Dermont Bouchard, Ph.D. Principal Investigator

_____Date_____
Tammy Jones-Lepp Branch Chief, ECB

_____Date_____
Margie Vazquez QA Manager, EMMD

Procedure for Determining Size and Zeta Potential Measurements on Zetasizer (4/4/2014)

- Use clean, dust free vessels for all work.
- Use DDI H_2O to rinse cuvette/cell before and after each samples.
- Close the lid, turn on the instrument and wait 30 minutes for the laser to stabilize.
- Use a clean, dust-free pipette place at least 1.0 mL sample to a clean and dust free quartz/disposable plastic cuvette (for size measurement) or 1.0 mL sample to the disposable capillary folded cell (for surface charge measurement).
- Insert the cuvette/cell. For the folded capillary cell, insert the cell with the front part towards the laser.
- When request, insert the cell into the instrument and wait for the temperature to stabilize.
- Make the measurement by clicking the START button using either manual method or using the pre-exist SOP method.

Quality Assurance/Quality Control:

- Run size standards at the beginning of each day before running samples:
    - Size – place 60 nm latex standard in bath sonicator for 15 minutes.
    - Dispense 1 drop of the latex standard into waste container (sacrifice first drop) and then add 2 drops to 20 mL of 10mM NaCl solution that has been filtered through a 0.1u prewashed (pre-wash protocol is a 50 mL DDI wash) Anotop filter. Note – filtering is for NaCl solution before adding Latex standard.
    - Swirl standard suspension to ensure complete mixing and re-sonicate for 15 minutes.
    - Using a clean, dust-free pipette place 1.5 mL of the dilute Latex solution into a clean and dust free quartz/disposable plastic cuvette and measure size in Zetasizer.
    - If the size is in the acceptable range (60 ± 5 nm), proceed with samples (approx. 1.5 mL).
    - If size is outside of acceptable range consult research leader.

- Run zeta potential standards at the beginning of each day before running samples:
    - Place approximately 1 mL (filling the cell) of zeta potential standard into a capillary folded cell. Make sure no bubbles are inside the cell before placing into the sample chamber. If there are bubbles, gently tap on the bench top to remove the bubbles.
    - If the zeta potential is in the acceptable range (50 ± 5 mV), proceed with sample measurement.
    - If the zeta potential is outside the range, check the disposable capillary folded cell. If the electrodes inside the cell become blacken, discard it and use a new one. If using a new cell, first rinse with methanol, then thoroughly with DDI H_2O.
- The concentration of samples should not be too low (> 0.1 mg/L and 0.1 g/L for particles with size from 10-100 nm and particles with size from 100 nm to 1 μm, respectively) or too high (< 5% and 1% mass L for particles with size from 10-100 nm and particles with size from 100 nm to 1 μm, respectively).

References:

[1] Dahneke, B.E. (ed) Measurement of Suspended Particles by Quasielastic Light Scattering, Wiley, 1983.

[2] Xiaojun Chang, W. Matthew Henderson, and Dermont C. Bouchard. Multiwalled Carbon Nanotube Dispersion Methods Affect Their Aggregation, Deposition, and Biomarker Response. *Environ. Sci. Technol.* **2015,** *49*, 6645-6653.

Protocol for Making Titration Measurements

SOP# RSB-012.1 effective 3/31/2009, revised 6/11/15

Exposure Methods and Measurement Division
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Athens, GA

Concurrences:

_____Date_____
Dermont Bouchard, Ph.D. Principal Investigator

_____Date_____
Tammy Jones-Lepp Branch Chief, ECB

_____Date_____
Margie Vazquez QA Manager, EMMD

Procedure for Making Titration Measurements (3/31/2009)

Setup

- Filling the titrant containers (not Fill tab)
    - Titrant volumes: 5-25 ml.
    - Specify the titrants concentrations.
    - Concentration of 0.25 M is satisfactory for most measurements. If a large number of points is to be used, a lower concentration may be needed.
    - For a wide pH range, it is useful to use two concentrations of titrant, e.g. 0.25 M of acid and 0.01 M of the same acid.

- Cleaning as shown in the following

- Priming the titrant syringe pumps and tubes
    - Prime the dispenser every morning or at the beginning of a new session
    - Always prime the unit when a titrant is changed.
    - 3 prime cycles will be sufficient to remove previous titrant
    - To eliminate the effect of surface tension, attach a sample container containing clean water to the dispersion head.

- Calibrating the pH probe
    - Calibrate before each titration session
    - At least 2 pH buffers
    - A container of clean DDI to rinse the probe between measurements

Measurement

- Sample volume: 10 ml (5 ml minimum to 25 ml maximum)
- Connecting the cell tubing
- Fill the cell with stirrer control (40% of normal speed)
- Titration sequence

Cleaning

- Replace the sample container with cleaning fluid (DDI H2O)
- Click "Clean" button with 3 cycles.
- Enable stirrer and pumping air into the tubing by removing the cleaning fluid while leaving the pump running

Quality Assurance/Quality Control:

- **pH probe**
    - pH probe operates over a range of pH 1 to pH 14 and temperature range of 0 to 70°C, though it is recommended that the titrations are performed at ambient temperature conditions by setting the measurement temperature equal to ambient temperature.

- It is recommended that the pH probe is calibrated before each titration session using at least two buffers. Maximum four buffers can be used for calibration. The pH probe should be recalibrated if the room temperature changes by more than 5°C.
- When the pH probe is immersed in a buffer solution, the pH should be attained (to within 0.1 of the pH) within 10 seconds. If this time exceeds 30 seconds, and the probe cannot be recovered, then the probe should be replaced. When changing the sample container, do not allow the probe to be exposed to air for more than 20 seconds.

- **Samples and Titrants**
  - An initial sample volume of 10 mL is recommended (5 mL minimum to 25 mL maximum). The sample used should be of a suitable concentration that gives an intensity of scattering within recommended value in Zetasizer Nano. This is checked at the beginning of each titration. If a message occurs indicating the sample concentration is higher than recommended, dilute the sample and restart the titration.
  - The ends of the sample exit and return tubes must always be below the surface of the sample. The ends of the titrant tubes must always be below the surface of the sample.
  - Filling the cell should be performed at a lower speed (40%) than during a normal titration to prevent bubbles.
  - The titrant containers will hold up to 25 mL of titrant. When the level reaches approximately 5 mL, it is recommended to re-fill the container.
  - The titrant concentration specified within the Titrants SOP dialogue must be accurate to within 10%.
  - The dispenser unit should be primed every morning or at the beginning of a new session.

References:

[1] Potential Sources of Error in Titration, UserCom 9 METTLER TOLEDO, August 2004.

[2] Fundamentals of Titration, METTLER TOLEDO 1998, Pages 137-140

Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Protocol for Dispersing C60 in water w/o THF or other organic solvents

Number: RSB-013.0	Effective Date: 3/31/2009

SOP was Developed X In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Dermont Bouchard

Signature: Date:

Approval

Name: Tammy Jones-Lepp
Title: ECB Branch Chief

Signature: Date:

Concurrence*

Name: Margie Vazquez
Title: EMMD QA Manager

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

* Optional Field

Protocol for Dispersing C_{60} in Water w/o THF or other Organic Solvents
Based on Brant et al. method in Langmuir 2006, 22, 3878-3885

1. Add 25 mg of powdered C_{60} per 100 ml DDW to an Erlenmeyer flask.

2. Mix with a magnetic stirrer at approximately 500 rpms for 1 week or longer.

3. Suspend stirring for 1 hour and then collect 10-20 mL aliquots from approximately 1-2cm below suspension surface.

3. Filter the suspension aliquots through a 0.45u cellulose acetate syringe filter; filtrate should be of a yellowish color. Combine aliquots to generate a volume of suspension adequate to complete an entire body of study.

4. Final C_{60} concentration should be about .5-5 mg L-1

5. Store these aqu/*n*C_{60} suspensions in refrigerator when not in use

Note: while developed originally for C_{60} fullerene, this method has been used with success for C_{60} derivatives, such as PCBM.

U.S Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
National Center for Computational Toxicology
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Protocol to Use Atomic Force Microscope Nanoparticles Imaging (ScanAsyst Air Mode)

Number: RSB-	Effective Date: 02/20/2014

SOP was Developed X In-house ☐ Extramural

Alternative Identification:	
SOP Steward	
Name: Dermont Bouchard Signature:	Date:
Approval	
Name: Tammy Jones-Lepp Title: Branch Chief ECB Signature:	Date:
Concurrence*	
Name: Margie Vazquez Title: EMMD QA Manager Signature:	Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

* Optional Field
NERL-SOP.2(11/2005)

- Sample preparation: Deposit nanoparticles onto surfaces of interest (e.g. silica wafer, mica, QCM-D sensors, and etc.) via drop casting or QCM-D deposition.
- Turn on the NanoScope V controller after turning on the computer.
- Sample loading: Attach the sample to a 15 mm metal disk using double side tape and mount the sample puck atop the scanner tube. An internal magnet will hold the puck down.
- Installing the head: With the sample in place, mount the head by gently lowering it over the scanner tube while checking for clearance. The top of the sample should protrude no more than several millimeters above the top of the head's X-Y translation stage. Secure both retaining springs and plug the head's connector into

the supporting ring.

- Loading a Probe: Turn the tipholder upside down with the groove facing up; Apply gentle downward pressure to lift spring clip; Slide the ScanAsyst-Air probe carefully into the tipholder's groove; Locate probe flush against inside edges of groove.
- Installing the Tipholder: Verify that the head is sufficiently raised to clear the sample with the tip. Insert the loaded AFM tipholder into the MultiMode's head by lifting the tipholder carefully over the sample. Press gently forward, then lower the tipholder. Secure tipholder using clamping screw at rear of the head.
- Laser Alignment: Adjust the laser position using vertical and horizontal adjustment knobs to align the laser on the tip of cantilever; Move the mirror lever on the back of the head to maximize the SUM (total voltage generated by the photodetector) signal; Adjust the photodiode positioned to set the photodetector output signal (RMS AC volts) and the deflection voltage to ~0.
- Starting imaging: Turn on the NanoScope 9.0 software and choose ScanAsyst-ScanAsyst Air mode; Bring the probe close to sample surface using the toggle switch on the MultiMode base; Turn on the Set ScanAsyst Auto Control to "on" and engage the probe to sample; once it is engaged, change scan size, aspect ratio, tip velocity, and other parameters as needed; Click the "capture image" button and the software will save the image automatically after each scan.
- Stopping imaging: Withdraw the probe from sample surface for five times (20 μm for each time); Then bring the sample surface away from probe using toggle switch; Remove the tip holder after it is released by loosening the clamping screw; Remove the sample; Turn off the software; And turn off the controller.

National Exposure Research Laboratory
National Center for Computational Toxicology
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Protocol to Run Samples on QCM-D	
Number: RSB-	Effective Date: 03/12/2010
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Alternative Identification:	

SOP Steward

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- Prior to the experiments, clean the crystal sensor using the cleaning protocols

mentioned in QCM-D manual. Cleaning protocol is different for different sensor surfaces.

- Switch the electronics unit on, and start QSoft 401. Wait a few seconds for the program to establish connection to the instrument. When "Tact" shows a value on the information bar below the main screen, the connection is established.
- Click on the temperature info bar to access the **Temperature Control** settings window. Setup the desired temperature for the experiment.
- Connect tubing and pump to the flow modules.
    - Before inserting a sensor crystal, make sure that the o-ring is lying flat in its track.
    - Insert the sensor crystal with backside electrode positioned as the symbol indicates; close the flow module (finger tight) and place on chamber platform.
    - For liquid measurements, fill the system with the start solution/buffer and make sure no air is introduced.
    - Note the numbering of the sensor positions.
- In the Qsoft main menu, go to "Acquisition" and then "Setup Measurement" and pick the sensor crystals corresponding the chosen platform positions and overtones to be measured, by ticking the boxes.
    - For standard measurements, make sure "Automatically optimize all resonances" are checked.
    - Start searching for the resonances of each sensor crystal by clicking "Find all". D values in water should be within 20% from the typical values for 1st overtone 357.8 and 3rd overtone 186.2. D values in air should be for 1st overtone 30 and 3rd overtone 15. A larger deviation from these values indicates:
        - The crystal might be incorrectly mounted in the flow module- check carefully that it is lined up and lies flat. A good sensor should show clean peaks.
        - The crystal might be damaged- try a different crystal.
        - There is a trapped air bubble over the sensor surface.
- Start measuring by clicking "Start Measurement" under "Acquisition" in the main Qsoft main menu.
- At the end of the experiment, clean the system:
    - Mount a sensor surface into the flow module.
    - Pump approximately 20 mL of 2% Hellmanex II through the system (heating to 30 ^{o}C could improve the cleaning effect).
    - Pump 100 mL of milliQ water through the system.
    - Empty the flow module from liquid and dry visible parts with nitrogen gas.