8th International Congress of Nanotoxicology, Boston June 1-4, 2016

FIRST ANNOUNCEMENT

WORKSHOP:

"Assessing the dose of nanomaterials in toxicological studies: Advanced approaches utilizing experimentation and fate and transport modeling"

Chairs: Philip Demokritou, Ph.D.; Flemming Cassee, Ph.D.
Instructors: Joel M. Cohen, Sc.D.; Sandra V. Pirela, Sc.D.; Glen M. DeLoid M.D.

Objective:

The objective of this workshop is to review the tools currently available to nanotoxicologists for quantifying particle dosimetry in both *in vitro* and *in vivo* experimental models. This includes a series of brief lectures that will provide the necessary background information/concepts and overview of methods in addition to hands-on training of both experimental and computational approaches for *in vitro/in vivo* dosimetry. Participants will be trained using a number of relevant real world case studies that demonstrate the applications of the emerging methods presented here.

Registration:

This "hands-on" training will be offered free of charge to registered conference participants. However, seats are limited to 30 people. Interested parties need to apply by email to the workshop chair, Dr. Philip Demokritou (pdemokri@hsph.harvard.edu), no later than on May 1st. Accepted participants will be informed by email of their enrollment in the workshop. Please note that ONLY accepted participants will be allowed to take part in the workshop.

Introduction:

In vitro high-throughput screening platforms based on mechanistic injury pathways have been used for hazard assessment of engineered nanomaterials (ENMs). Toxicity screening and other *in vitro* nanotoxicology assessment efforts typically compare and rank the bioactivity of nanomaterials relative to each other. It has been shown that ENMs hazard rankings are highly sensitive to variability in poorly standardized dispersion protocols and lack of dosimetry. This sensitivity is largely due to the impact of particle transformations on partico-kinetics that affect bioactivity and delivery of particles to cells. The

importance of such dosimetric considerations apply to studies using both cell cultures as well as animal experimental models for toxicology assessments. Thus, extrapolation to humans becomes more reliable once the dose is determined accurately at the site of deposition.

Emerging hybrid, experimental/computational approaches to cellular dosimetry can be used by nanoparticle toxicologists to accurately calculate the delivered to cell dose metrics for various ENMs and *in vitro* experimental conditions as a function of exposure time. In addition, *in vivo* lung deposition models allow researchers to estimate the delivered particle dose in any region of the respiratory system, as well as study the implications of particle properties and breathing parameters. More importantly, such dosimetric methodologies enable nanoparticle toxicologists to bring *in vitro* and *in vivo* doses to the same scale, an important step towards the development and validation of *in vitro* cellular screening assays.

Required Materials:
Laptops with Microsoft Excel and MATLAB are recommended for this workshop. While a limited number of PCs will be available for use, participants are encouraged to bring their own. Please also download the MPPD model ahead of time if possible (click **here** to download).

Workshop handouts will be provided on a USB storage unit and will include the following:

- presentation slides, relevant research articles, source code for Harvard Distorted Grid (DG) *in vitro* dosimetry model
- manual instructions for volumetric centrifugation method (VCM) for measurement of effective density of ENMs in liquid suspensions
- case study dispersion preparation and characterization data for 3 ENMs for in-vitro dosimetric analysis: fractal nano metal oxide, gold nanospheres, buoyant nanoparticles.
- data for dispersion characterization values necessary for *in vitro* dosimetry DG modeling
- human exposure characterization data for MPPD modeling to calculate dose delivered to the lungs and equivalent cellular dose *in vitro*
    - case study: laser printer-emitted engineered nanoparticles (PEPs)

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Introduction		

1:30 – 2:15	An overview of emerging tools for the *in vitro* and *in vivo* dosimetry of engineered nanomaterials (Philip Demokritou, Flemming Cassee)	• From exposures to *in vivo* and *in vivo* dosimetry
		  ○ exposure characterization
		  ○ estimation of deposited dose in the lung (for inhalation route) using modeling (MPPD) (Dr Cassee)
		• Overview of the *in vitro* dosimetry continuum and available methodologies (Dr Demokritou)
		  ○ Step 1: ENM dispersion preparation
		  ▪ sample collection and characterization
		  ▪ calorimetric calibration of sonication equipment
		  ▪ sonication protocols
		  ○ Step 2: ENM dispersion characterization
		  ▪ hydrodynamic diameter, polydispersity, zeta potential, conductivity, effective density, stability
		  ○ Step 3: computational fate and transport modeling for *in vitro* dosimetry (i.e ISDD, VCM-ISDD, Harvard DG, CFD)

Dosimetry Workshop Part A: Dispersion preparation and characterization for *in vitro* studies (Steps 1 & 2)		
2:30-3:00	Overview of standardized protocols on ENM dispersion preparation and characterization (Drs Joel Cohen, Sandra Pirela)	• Calorimetric calibration of sonication equipment • Overview of dispersion preparation protocol • Overview of colloidal characterization ○ Available Instrumentation to measure: Hydrodynamic diameter, polydispersity, zeta potential, conductivity, stability, effective density
3:00 – 3:15	Introduction to the Harvard Volumetric Centrifugation Method (VCM) for effective density measurements (Drs Joel Cohen, Sandra Pirela)	• Overview of full VCM protocol • Summary of relevant articles, applications, and implications for *in vitro* hazard ranking

3:15 – 3:45	Hands-on VCM training (Drs Joel Cohen, Sandra Pirela)	• Hands on measurement of suspended nano agglomerates with pre-centrifuged packed cell volume (PCV) tubes for 3 case study samples:
		  ○ fractal agglomerated engineered nano metal oxide
		  ○ monodisperse, non agglomerating, gold nanospheres
		  ○ buoyant nanoparticles
		• Workshop session on how to calculate effective density from VCM measurement for 3 case study particles
		  ○ Selection of appropriate stacking factor value
		  ○ Determination of necessary inputs for effective density calculation
		• Calculate the effective density for all 3 case study samples

3:45 - 4:00	Break	
Dosimetry Workshop Part B: Computational fate and transport modeling for *in vitro* dosimetry (Step 3)		
4:00 – 4:20	Introduction to fate and transport modeling (Dr Glen DeLoid)	• Numerical tools available for *in vitro* fate and transport

		modeling for formed agglomerates
4:20 – 5:00	Hands on training with Distorted Grid (DG) model for *in vitro* dosimetry (Dr Glen DeLoid)	• Demonstrate how to calculate volume-weighted hydrodynamic diameter from steps 1 & 2 for the 3 case ENM studies • Demonstrate how to run the DG model in MATLAB • Participants will then use the model to calculate delivered mass, delivered particle number, delivered surface area per cm^2 for each of the 3 case study particles based on the measured effective density and volume-weighted hydrodynamic diameter
Dosimetry Workshop Part C: Using the Multiple Path Particle Dosimetry (MPPD) Model to translate inhalation exposures to delivered to lung dose: Matching *in vivo* with *in vitro* doses		
5:00- 5:25	Hands on training with Multiple Path Particle Dosimetry (MPPD) Model (Dr Sandra Pirela)	• Describe full protocol • Participants will run MPPD model based on data set for laser printer-emitted particles (PEPs) to determine mass flux in the lung and deposited dose metrics • Participants will use the results of the MPPD model and the DG model for this case study particle to determine *in vitro* dose levels equivalent to expected human exposure doses
5:25 – 5:30	Questions and session wrap-up	