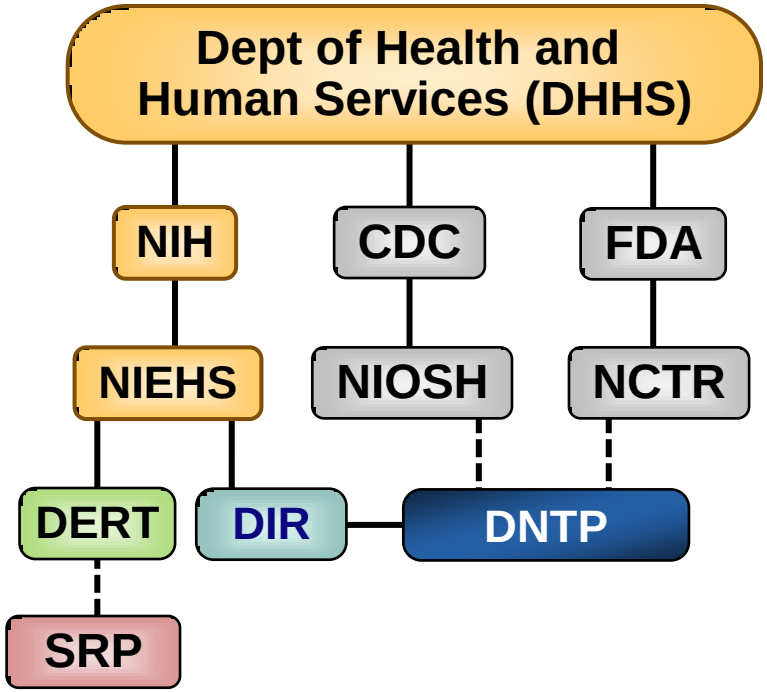
Nanotechnology Environmental Health and Safety Research Program at NIEHS

Srikanth Nadadur, Ph.D.

Division of Extramural Research and Training

NIEHS

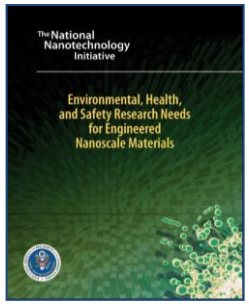
Research Triangle Park, NC

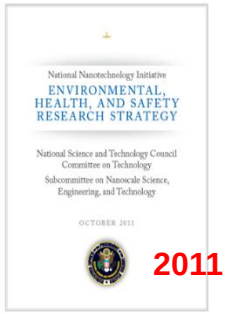


Mission: Reduce the burden of human illness and disability by understanding how the environment influences the development and progression of human disease.

NIH National Institute of Environmental Health Sciences

National Nanotechnology Initiative





2011

OSTP OMB

NIH NSF

DOE DOD

NIST FDA

NASA USDA

NIOSH USDA FS

DOS EPA

DOTr NRC

DOT USPTO

ITIC USGS

DOL

DOJ DOEd

DOC BIS ITC

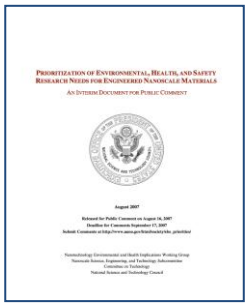
DHS CPSC

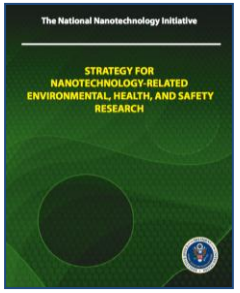
National Nanotechnology Initiative

Collaborative, Multi-agency, Cross-cut Program Among 25 Federal agencies

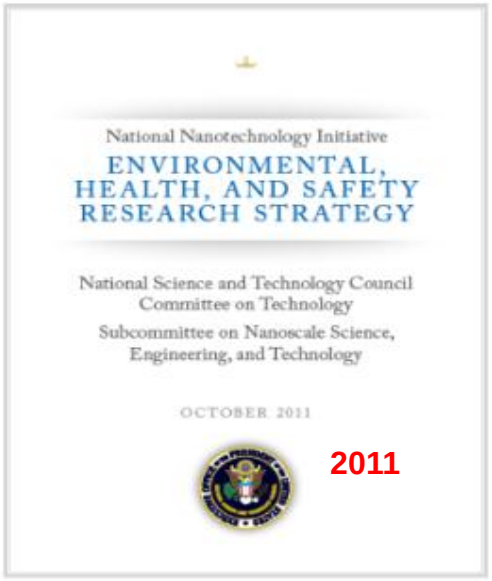
Coordinated support for fundamental R&D to advance nanoscale science for:

- **Global leadership**
- **National economic benefit**
  - **National security**
  - **Improved quality of life**





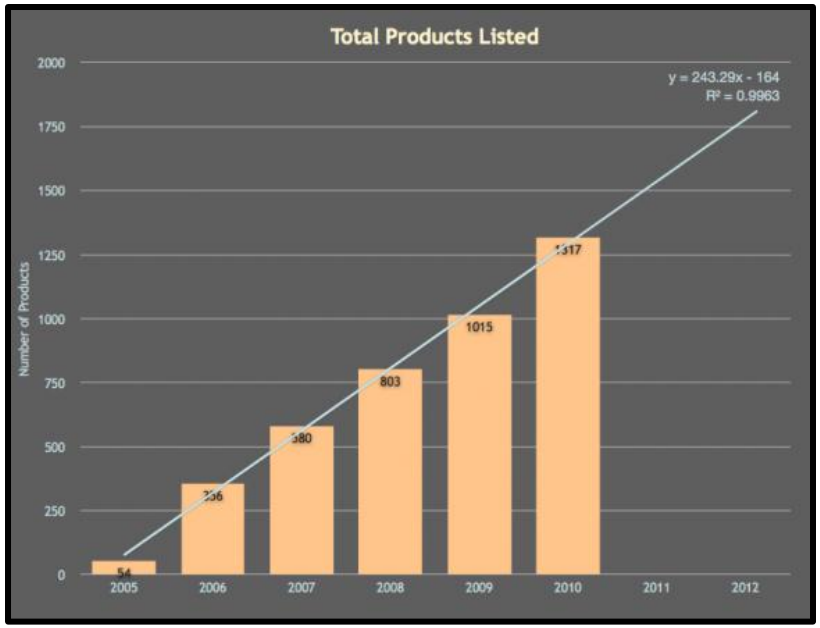
NNI Nano EHS Research Strategy : Focused areas

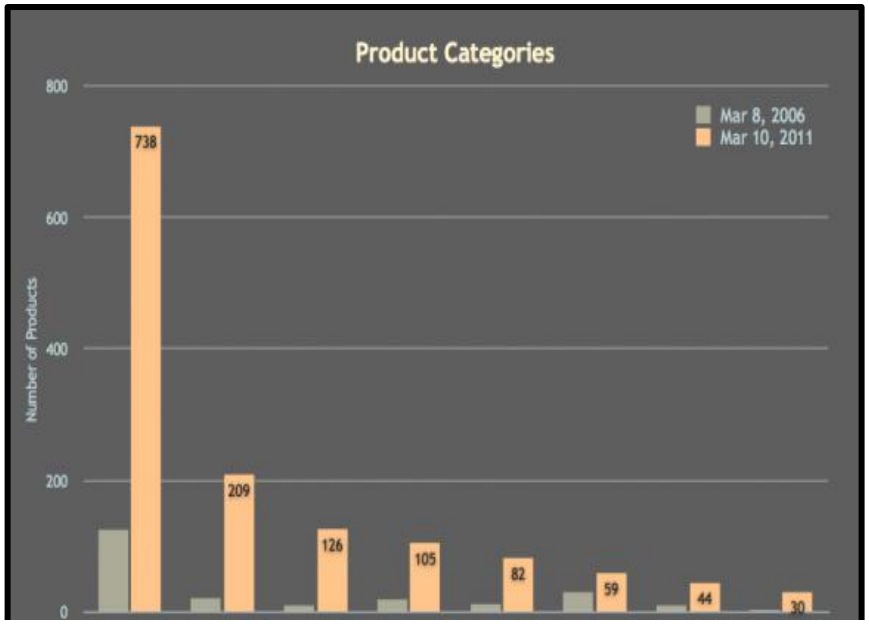


- Human Health
- Human Exposure Assessment
- Nanomaterial Measurement Infrastructure
- Environmental Effects
- Risk Assessment and Risk Management Methods
- Informatics and Modeling for Nano EHS Research

Nanotechnology Based Products

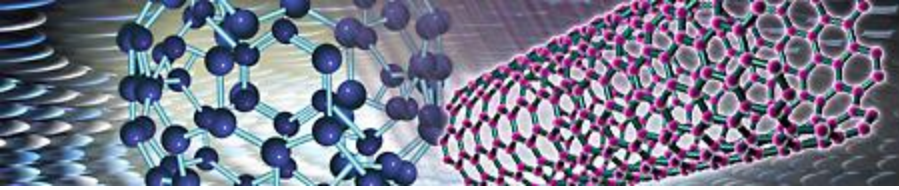
~2,800 products in market as of 2012





Major ENMs: Silver (313),
Carbon (90), Titanium (59)
Silica (43), Zinc (31) and Gold (28).

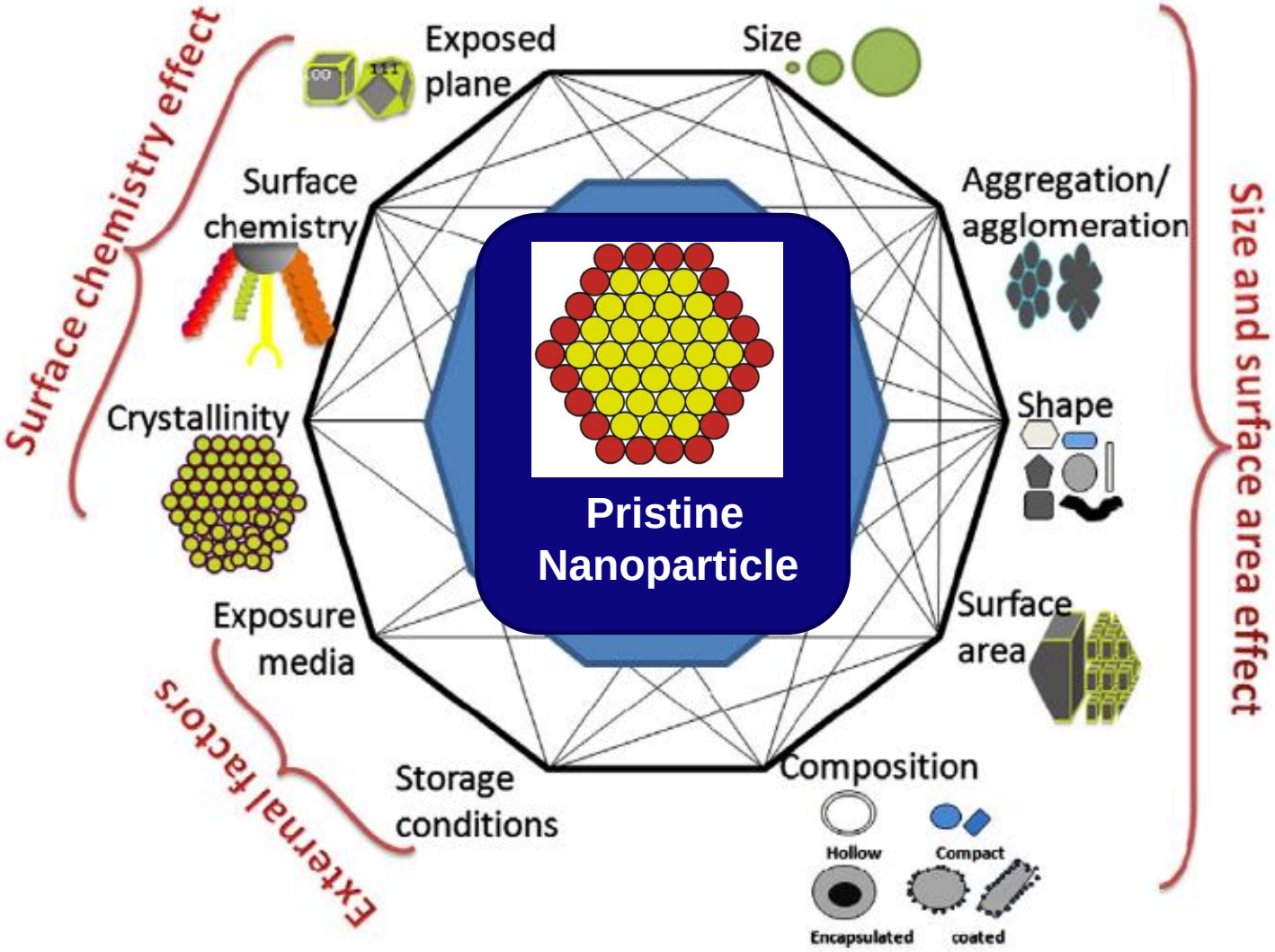
http://www.nanotechproject.org



Nano EHS Challenges

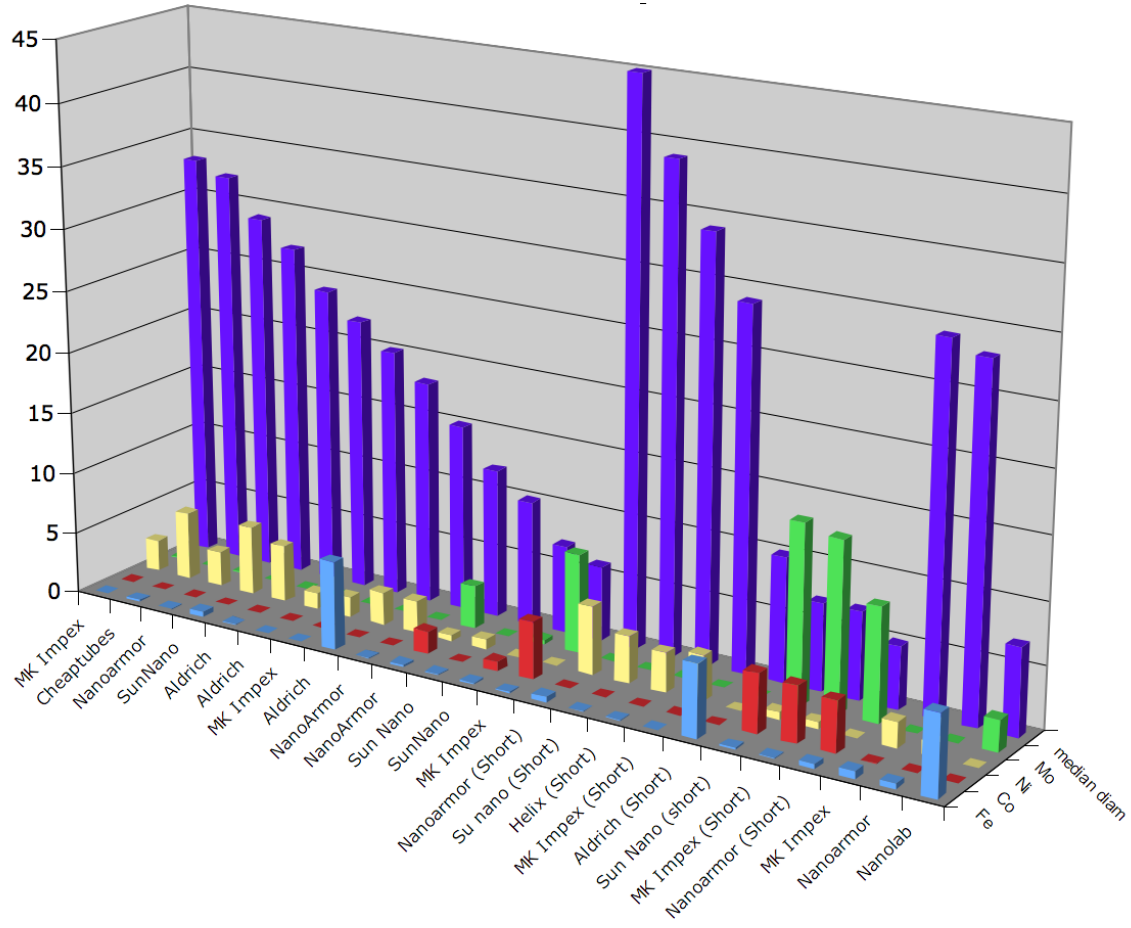
- Near infinite number of Engineered Nanomaterials (ENMs) can be generated from any metal, non-metal and organic compounds with a defined size, structure and shape.
- Potential health effects are not known
- Production methods affect properties of ENMs
  - Similar particle may exert different health effects
- Humans may be exposed to ENMs through multiple routes
  - Inhalation, dermal, ingestion, other portals of entry
- Need a defined metric to assess toxicity
- Identification of potential hazard associated with specific physical and chemical properties is critical in guiding safer development and use of nanotechnology

ENMs - Dissolution

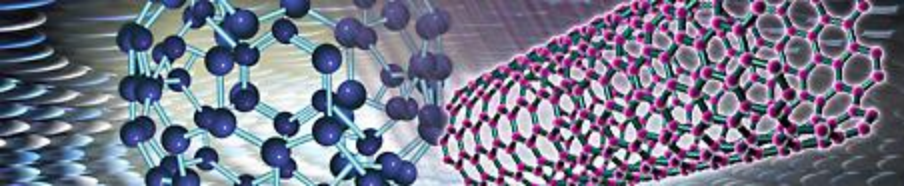


Adapted from Misra et al 2012

Grappling With the Challenge of Characterization: MWCNT Mixtures of Diameter, Length and Metals



Walker, 2011, personal communication



ENMs Cytotoxicity Assays

Cytotoxicity assay	Detection principle	NP interference	Altered readout	Particle/Reference
Cell viability / MTT	Colorimetric det. of mitochondrial activity	Absorption of substrate	Cell viability	Carbon NP/Worle-Knirsch (2006), Belyanskya (2007), Monteiro-Riviere (2006)
Neutral red	Colorimetric det. of intact lysosomes	Dye adsorption	Cell viability	Carbon NP/Casey (2007)
LDH	Colorimetric det. of LDH release	Inhibition of LDH	Cell necrosis	Trace metal-containing NP/Suskaa (2005), Pulskamp (2007)
Annexin V/ Propidium Iodide	Fluor. Det. of PS PI staining of DNA	Ca^{2+}-depletion Dye adsorption	Apoptosis/ necrosis	Chitosan NP/ Trotter (1995) Carbon NP/ Shukla (2005)
Caspase	Fluor. det. of caspase 3 activity	Inhib. of Cas-3	Apoptosis	Trace metal-containing NP/Stennicke (1997)
Stress Response DCF	Fluor. det. of ROS	Fluor. quenching	Oxidative stress	Carbon NP/Aam (2007)
Inflammatory response/ ELISA	Colorimetric det. of cytokine secretion	Cytokine adsorption	Cytokine concentration	Carbon NP/ Monteiro-Riviere (2006) Metal oxide NP/ Veranth (2007)

Modified from Kroll et al., E. J. of Pharm. & Bio., (2009)

2011

NNI Nano EHS Research Strategy (Human Health)

1. Identify or develop appropriate in vitro and in vivo assays and models to predict in vivo human exposure responses to ENMs;

2. Quantify and Characterize ENMs in exposure matrices and biological matrices;

3. Understand relationship between ENM physicochemical properties (PCPs) and their transport, distribution, metabolism, excretion and body burden in human body;

4. Understand relationship between ENM-PCPs & uptake through the human port of entry tissues;

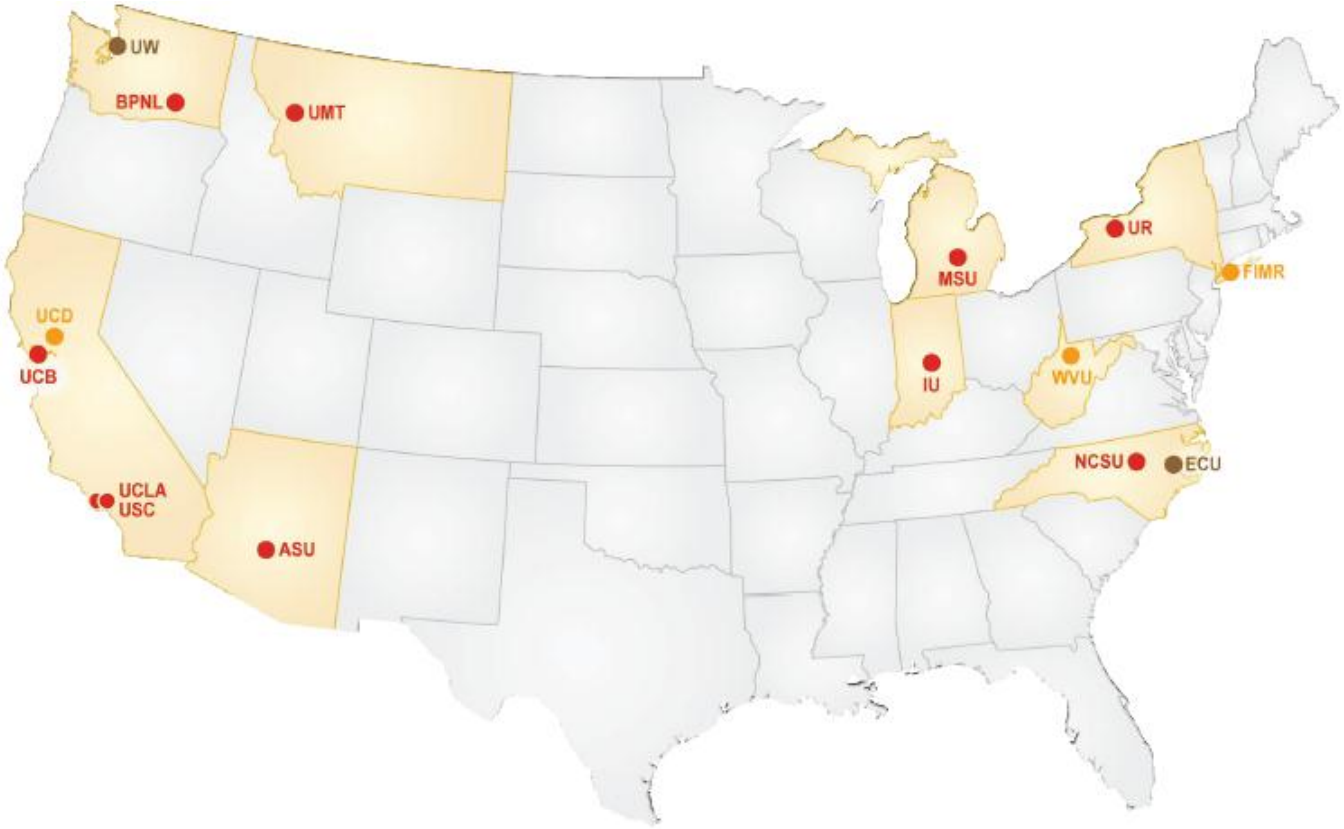
5. Determine modes of action underlying human bio-response to ENMs at molecular, cellular, tissue, organ and whole body levels;

6. Determine to extent to which life stage and/or susceptibility factors modulate health effects from exposure to ENMs or Nanotechnology Enabled Products and applications

NIEHS – Nano EHS Program

- Develop/identify relevant *in vitro* and *in vivo* assays to predict biological responses
- Gain fundamental understanding on interaction of engineered nanomaterials (ENMs) with biological systems- as dictated by their PCPs
- Develop predictive models to characterize health effects on exposure to ENMs
- Methods to quantify exposure to ENMs in diverse matrices
- Guide development of next generation ENMs with minimal adverse biological/health effects

Nano Grand Opportunity Consortium

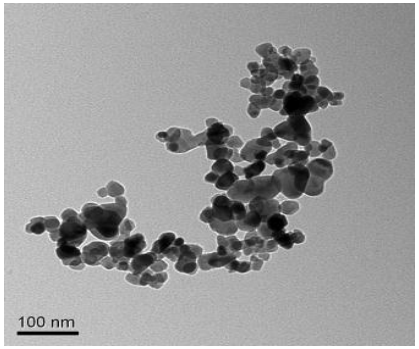


- Established Nano GO consortium with investigators from 15 institutions funded through ARRA in October 2009
- Approximately $13.75 M investment over 2 years

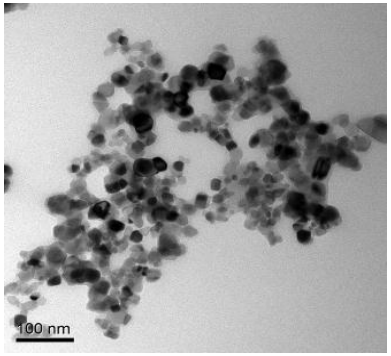
Goals of Nano Grand Opportunity Consortium

- Develop reliable and reproducible methods to assess biological response/toxicological endpoints for ENMs.- Round Robin efforts
  - Utilize ENMs with well defined physicochemical properties
  - Develop standardized protocols and methods for ENM dispersal and characterization in cell culture media.
  - *In vitro* and *in vivo* models that can reliably predict biological response and reproducible data across labs using well characterized ENMs
- Publish inter-lab round robin data in peer reviewed journal to be available for the scientific community as a reference document
  - Share how the consortium model allowed approaches to address technical issues with ease and improve the quality and reproducibility of data

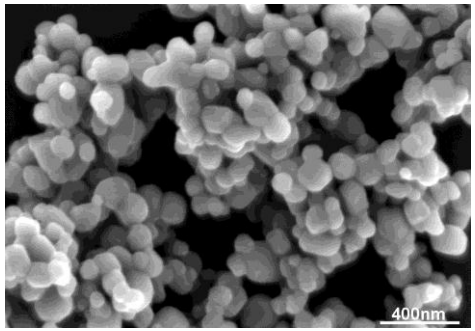
Nano GO: ENMs



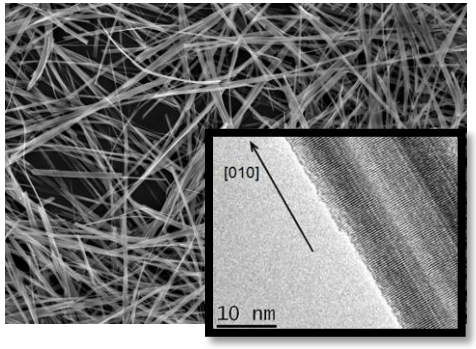
ZnO



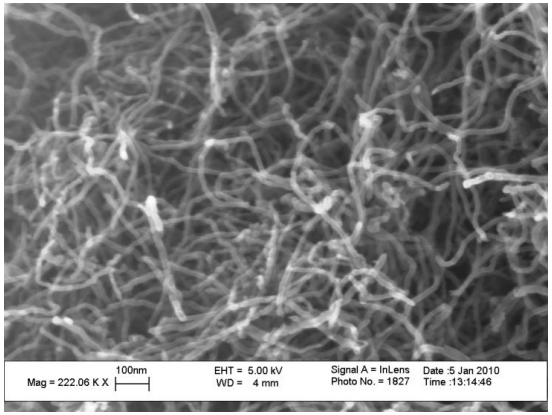
TiO2 P25



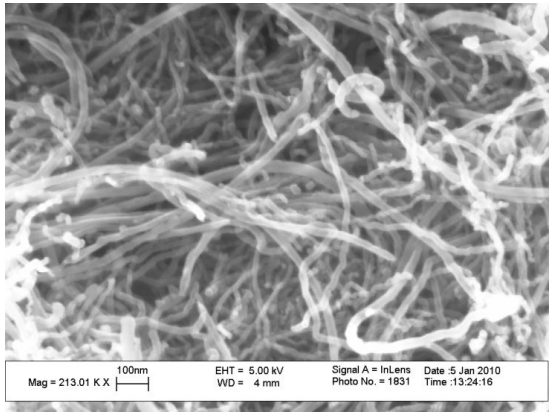
TiO_2 nanospheres (NS)



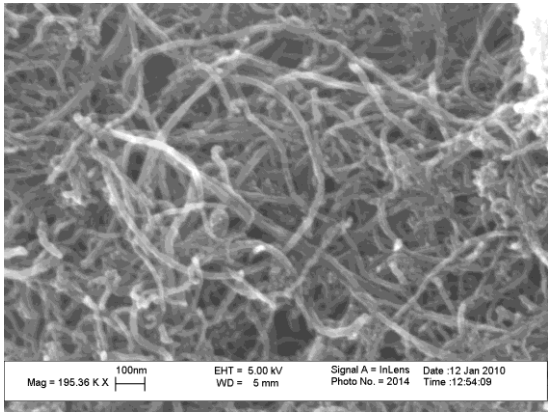
TiO_2 nanowires (NB-2)



Original MWNT



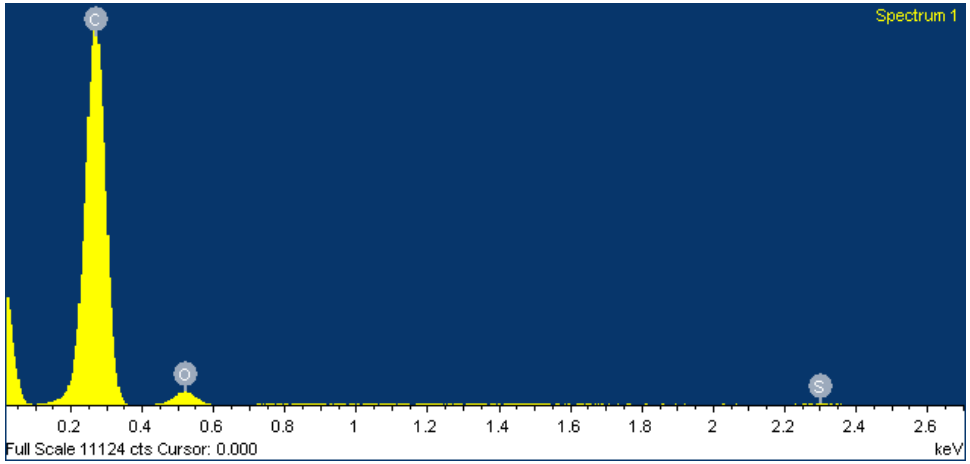
Purified MWNT



Functionalized MWNT

Outer Diameter – 20-30nm; Inner Diameter – 5-10nm; Length – 10-30µm

MWCNTs-Elemental Analysis



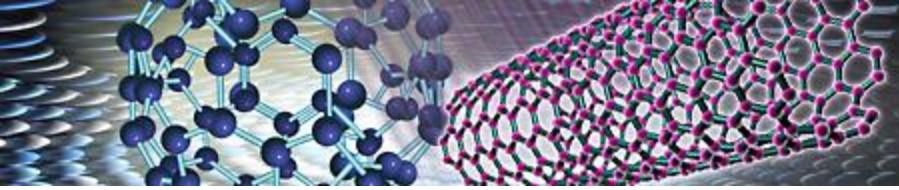
Functionalized MWNT

	Elemental Composition (%weight)		
	Ni	**Fe**	**S**
Original MWNT	**4.49**	**0.76**	**-**
Purified MWNT	**1.80**	**0.08**	**-**
Functionalized MWNT	**-**	**-**	**0.18**

5.27% COOH
0.03% SO_3

60% Ni removed with purification
90% Fe removed with purification

Mitra, NJIT



Manuscripts in Press- EHP

Inter-laboratory Evaluation of In Vitro Cytotoxicity and Inflammatory Responses to Engineered Nanomaterials of the NIEHS NanoGo Consortium

Xia, T., Hamilton Jr, RF, Bonner,JC, Crandall,ED, Elder, A, Fazlollahi, F, Girtsman, TA, Kim, K, Mitra, S, Ntim, SA, Orr, G, Tagmount, M, Taylor, AJ,, Telesca, D, Tolic, A, Vulpe, C, Walker, A, Wang, X, Witzmann, FA, Wu, N, Xie, Y, Zink, JI, Nel, A, and *Holian, A.

Nanoconsortium Interlaboratory *In Vivo* Evaluation of Rodent Pulmonary Responses to Engineered Nanomaterials

James C. Bonner[1*], Rona Silva[2], Alexia Taylor[1], Jared Brown[3], Susana Hilderbrand[3], Vincent Castranova[4], Alison Elder[5], Jack Harkema[6], Lori Bramble[6], Terrence Kavanagh[7], Dianne Botta[7], Andre Nel[8], Gunter Oberdorster[6], Dale Porter[4], and Kent Pinkerton[2]

NIEHS – Nano EHS Program

- Develop/identify relevant *in vitro* and *in vivo* assays to predict biological responses
- Gain fundamental understanding on interaction of engineered nanomaterials (ENMs) with biological systems
- Develop models to predict potential health effects on exposure to ENMs
- Methods to quantify exposure to ENM in diverse matrices
- Guide development of second generation ENMs with minimal adverse biological/health effects

NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR)

Administrative & Material Core

Project #1*: In Vitro*
Understand basic ENM-biological interactions (molecular, cellular, organelle, organ level). Diverse cell phenotypes, representing portals of entry

Project #2*: In Vivo*
Investigate how ENM PCPs influence physiological pathological outcomes in target/secondary organs; ADME, translocation across different organs

U01 Centers on In Vivo

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

NANOMATERIALREGISTRY

Risk Assessment

Project#3:
Risk Assessment Translation: Develop RA framework
In Two phases:
Phase1: conceptual framework
Phase2: Collaborative/integrated

NCNHIR Consortium

15 institutions in US & Imperial College London

★ U19 Centers
★ U01 Centers
★ Consortium

ENMs Investigated by NCNHIR

- C60 (native, radiolabeled)
- Metal oxides (24 - diverse shape, size, crystalinity, surface modifications)
- MWCNTs (commercially produced, purified, diverse surface modifications, aspect ratio)
- SWCNT (native, radiolabeled, surface modifications)
- Qdots (CdSe/ZnS; CdTe, diverse surface coatings)
- Metals (Silver, Gold, diverse shapes, sizes, aspect ratio)

NCNHIR Consortium: In Vitro Efforts

Target Organ System	Institution	Species		
		Mouse	Human	Rat
Pulmonary System				
Bronchial Epithelial (BEAS-2B)	UCLA		*	
Bronchial Epithelial (NHBE)	UCLA		*	
Alveolar Mac-Primary (+/- SRA)	PNNL	*		
Alveolar Mac- Primary	RESAC		*	
Lung Fibroblast- Primary	RESAC		*	
Alveolar type I (TT1)	RESAC		*	
Alveolar type II- Primary	RESAC		*	
Alveolar type II (C10)	ECU/PNNL	*		
Airway Epithelial- Primary	UW	*		
Tracheo/Broncho Epithelial- Primary	UW		*	
Immune System				
Monocyte/Mac (THP-1)	UCLA		*	
Macrophage (RAW 264.7, +/- SRA)	PNNL	*		
Mast cells - Primary (+/- SRA)	ECU	*		
Monocyte-Derived Macs- Primary	RESAC		*	
Cardiovascular System				
Aortic Endothelial- Primary (+/- SRA)	ECU	*		
Aortic Endothelial- Primary	ECU			*
Hepatic System				
Hepatocytes- Primary	UW	*		
Hepatocytes- Primary	UW		*	
Nervous System				
Neuronal (ED12 midbrain)	UW	*		
Neuronal (Differentiated Human ES Cells	UW		*	

Cell types: respiratory, liver, immune, neuronal, vascular systems; mouse and human

High Throughput Screening

NCNHIR Consortium: In Vivo Efforts

- Routes of Exposure:
  - IT, OP, gavage, IV, inhalation
  - Acute, Sub-chronic
- Gestational, lactational
- Zebrafish
- Diverse CC mouse strains
- KO (SR-A, ApoE)
- Disease models
  - Asthma, Emphysema
  - Infection
- Pulmonary toxicity
- ADME
- Developmental toxicity
- Cardiac toxicity
- Gut uptake, clearance
- Susceptibility
  - Genetic
  - Disease

NCNHIR Consortium ENMs

Phase 1:

- Nano-Silver was procured from nanoComposix, Inc.
  - 20 nm Ag spheres with and without Citrate/PVP coating
  - 110 nm Ag spheres with and without Citrate/PVP coating

Phase 2: Multiwall Carbon nanotubes
Three preparations: As procured, purified and functionalized

	OD (nm)	Length (μm)	Aspect Ratio
1	30-50	0.5-2	31
2	10-20	0.5-2	83
5	10-20	10-30	1333

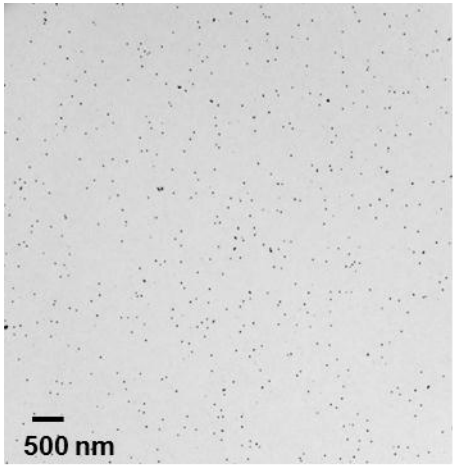
Major Biological Endpoints Represented

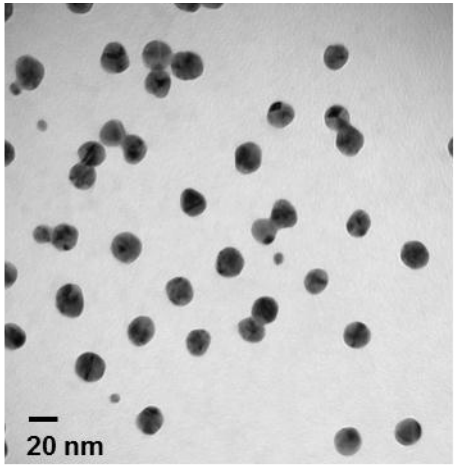
Institution	Species			Cell Viability	Cell Uptake / Localization	Inflammatory Markers	Oxidative Stress	Calcium Flux	Mitochon. Integrity	Bact. Phagocyt. &	Surfactant Composition	Protein Binding	Membrane Fluidity
	Mouse	Human	Rat										
UCLA		*		*		*	*	*	*				
UCLA		*		*		*	*	*	*				
PNNL	*			*		*	*			*			
RESAC		*		*	*	*	*		*	*	*		
RESAC		*		*	*	*	*		*		*		
RESAC		*		*	*	*	*		*		*		
RESAC		*		*	*	*	*		*		*		
ECU/PNNL	*			*	*	*						*	*
UW	*			*	*	*	*						
UW		*		*	*	*	*						
UCLA		*		*		*	*	*	*				
PNNL	*			*		*	*			*			
ECU	*			*	*	*						*	*
RESAC		*		*	*	*	*		*				
ECU	*			*	*	*						*	*
ECU			*	*	*	*						*	*
UW	*			*	*	*	*						
UW		*		*	*	*	*						
UW	*			*	*	*	*						
UW		*		*	*	*	*						

Endpoints associated with inflammation, oxidative stress and cell death (necrosis/Apoptosis); common modes of action under investigation across all centers.

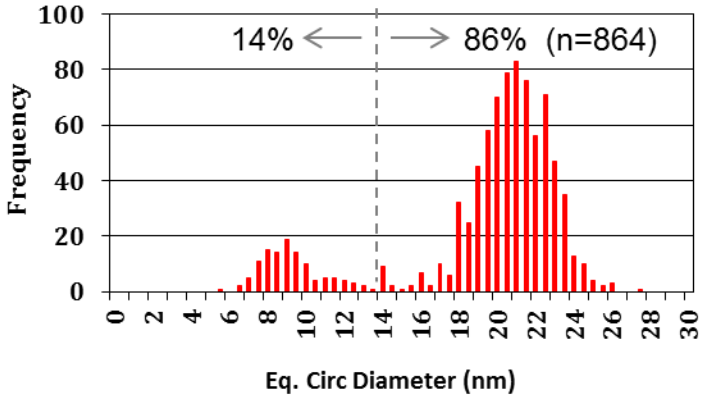
Silver 20 nm Spheres

20 nm Ag, citrate coated

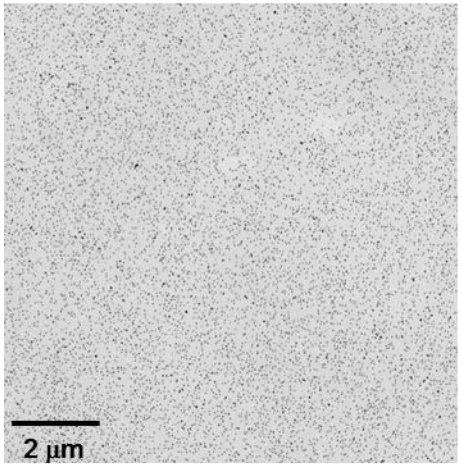


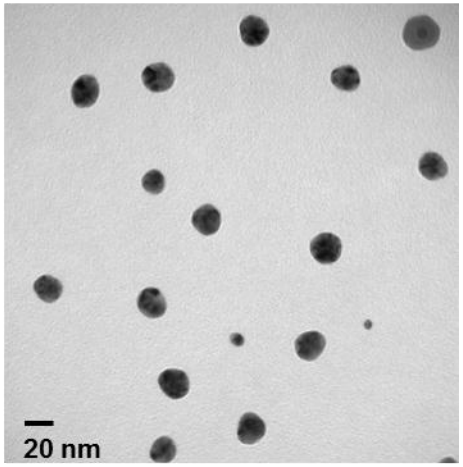


Average size = 20.8 ± 2.0 nm

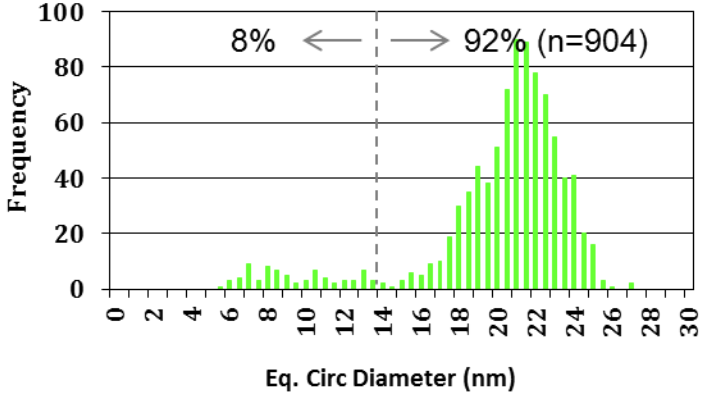


20 nm Ag, PVP coated



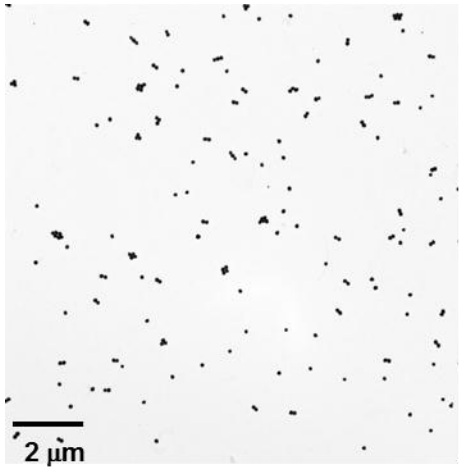


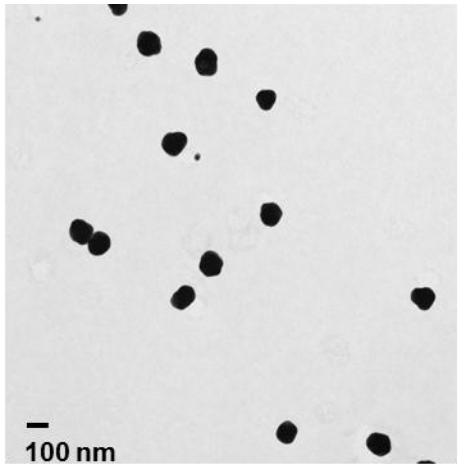
Average size = 21.2 ± 2.2 nm

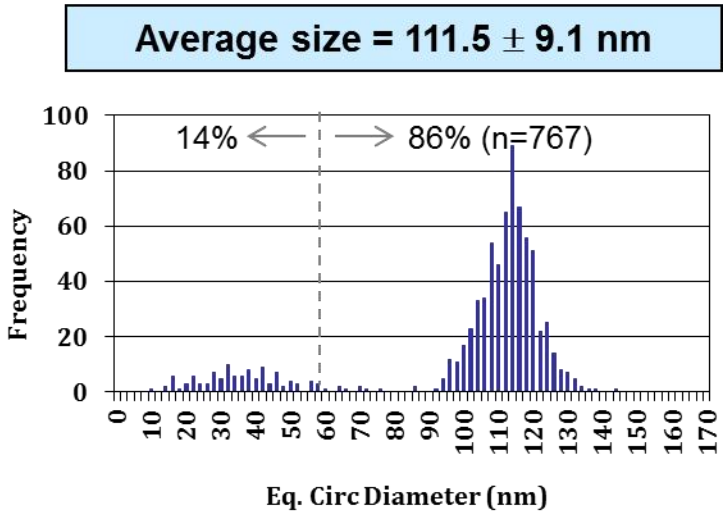


Silver 100 nm Spheres

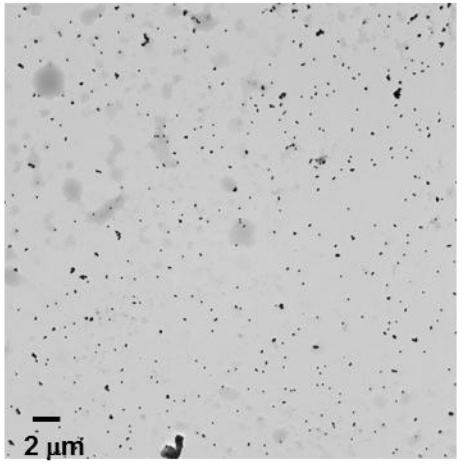
110 nm Ag, citrate coated

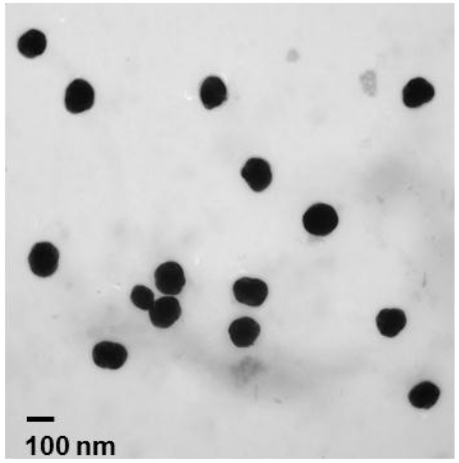


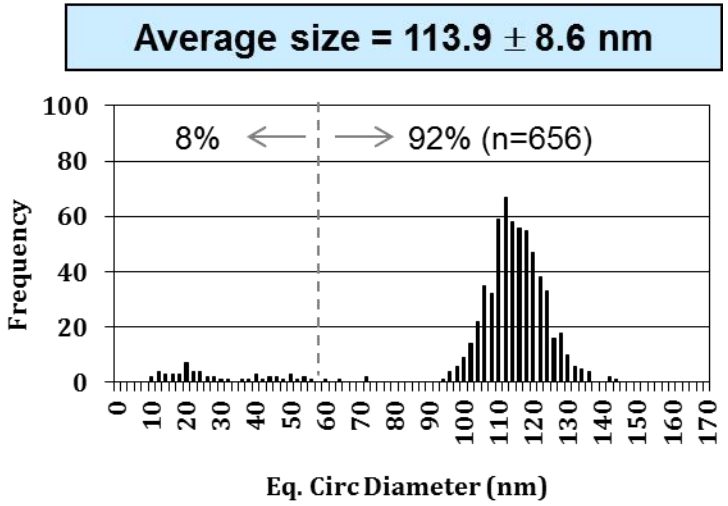




110 nm Ag, PVP coated







Based on Cytotoxicity Can we classify?

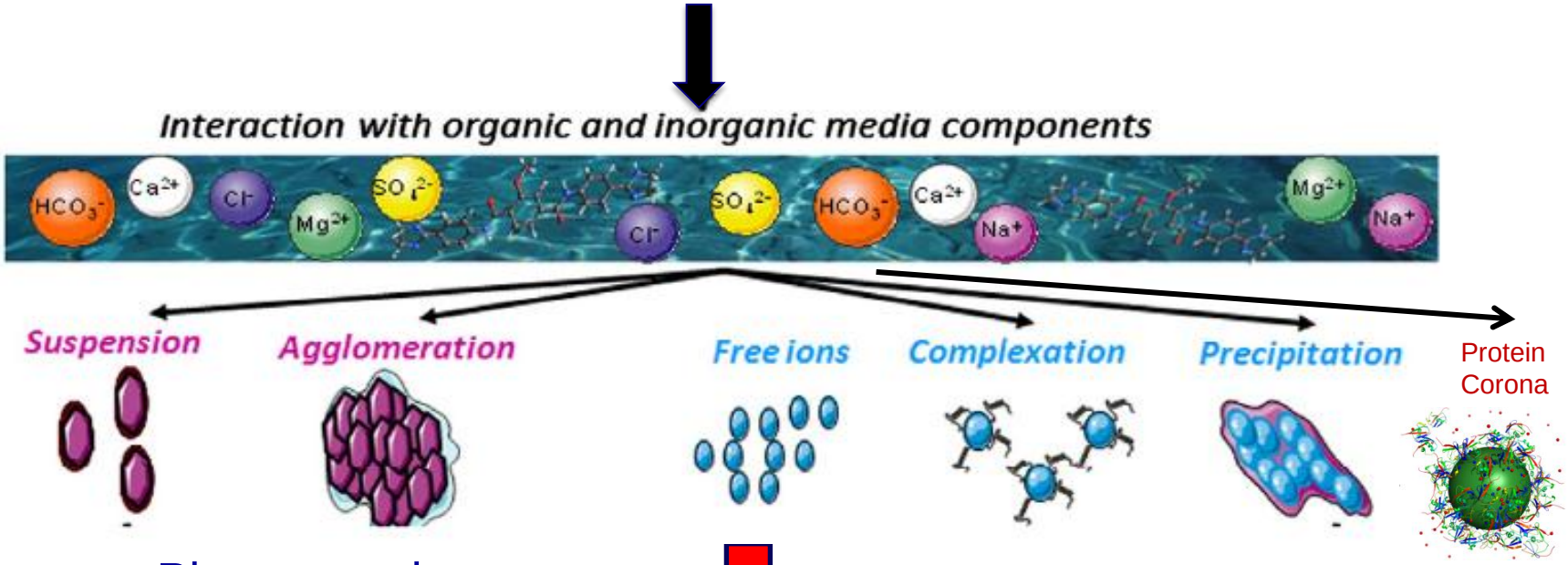
Susceptible

- THP1
- RAW264.7
- Mouse bone marrow derived macrophages
- Human monocyte derived macrophages
- Rat lung epithelial cells
- BEAS2B
- Alveolar type I
- Rat aortic endothelial cells
- Human vein umbilical vein cells
- Human hepatocytes
- Lung fibroblasts

Resistant

- Primary human alveolar macrophages
- Primary mouse airway epithelial cells (air-liquid interface)
- Primary human alveolar type II cells
- Mouse alveolar type II cells (C10)
- Caco-2 cells
- Mouse mast cells

Engineered nanoparticle

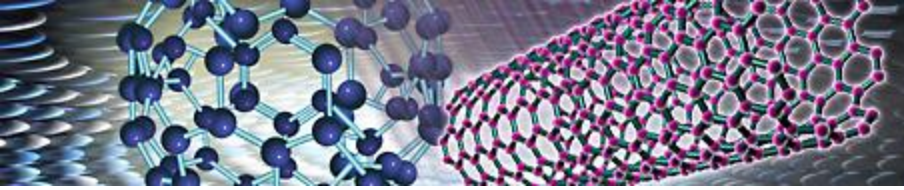


Phagocytosis
Endocytosis:
Caveolin/clathirin
Dependent/independent

Ion Channel
Ion transport

Cellular Uptake Mechanism

Adapted from Misra et al, 2012



In Vivo Studies

- Routes of exposure
  - Inhalation, IV, oral, dermal
- Diverse physiological states
  - Gestation, pregnant, lactational
- Pulmonary:
  - Inhalational exposure studies in rodents(rat, mouse)
  - Intratracheal instillation, Oropharyngeal aspiration
- ADME
- Cardiovascular
- Gastrointestinal
  - Influence on gut microbiome

Silver ENMs- Pulmonary Toxicity Studies

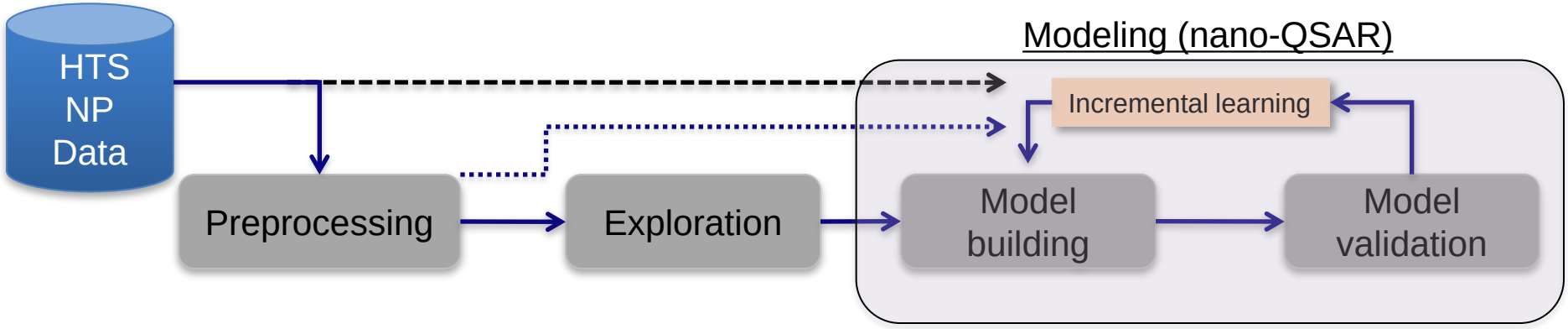
	Doses	Time Points
OPA	**Single 1 µg/g max/mouse (0.1, 0.25, 0.5, and 1 µg/g BW)**	**24 hrs; 7 days (21 days if warranted)**
Instillation	**Single 1µg/g max/rat (0.1, 0.25, 0.5, and 1 µg/g BW)**	**24 hrs; 7 days (21 days if warranted)**
Inhalation	**Single 1 mg/m^3 max**	**24 hrs; 7 days (21 days if warranted)**
IV		**24; 48 hrs**

Mouse Strains

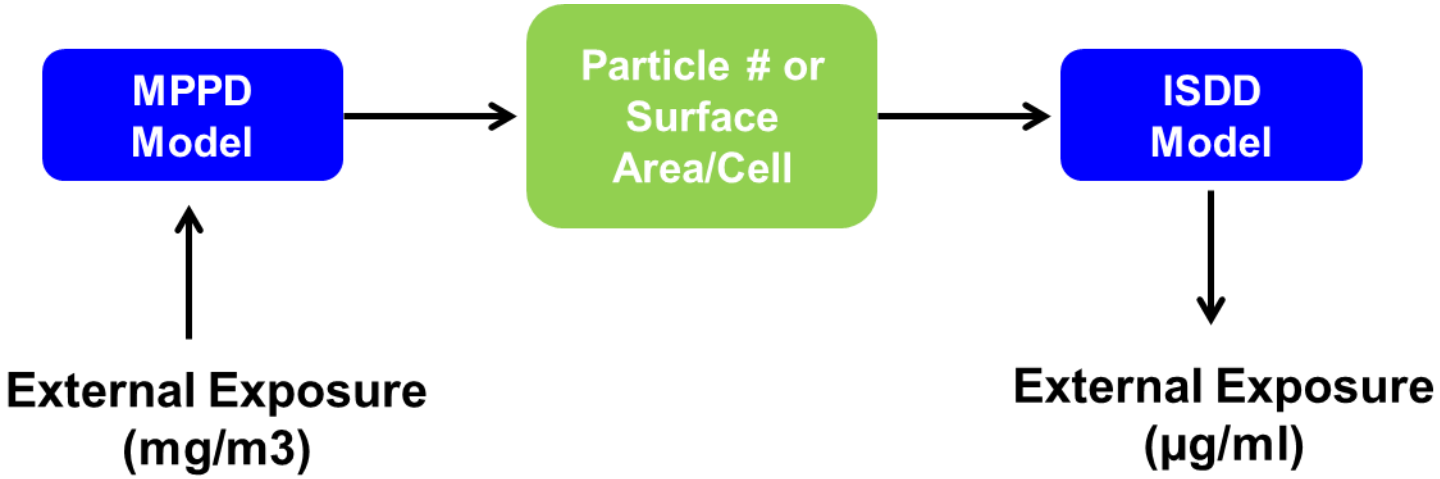
- UW – 7 strains
- NYU – 7 strains
- 0.25 µg/g 20 nm silver citrate
- 24 hr time point

- DATA
  - Control vehicle effect in certain strains
  - Strain responses for % PMNs and Protein

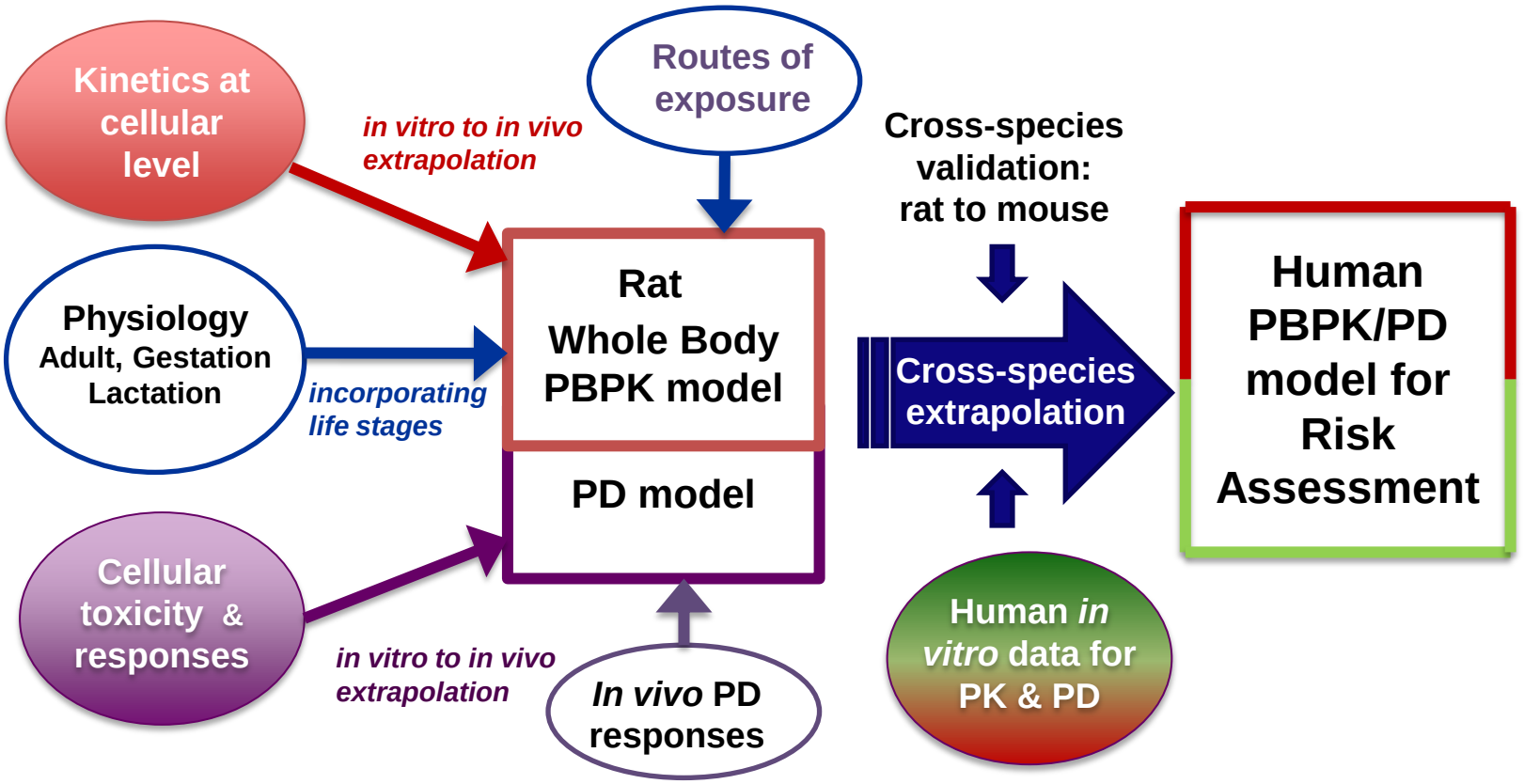
Predictive Modeling: QSAR



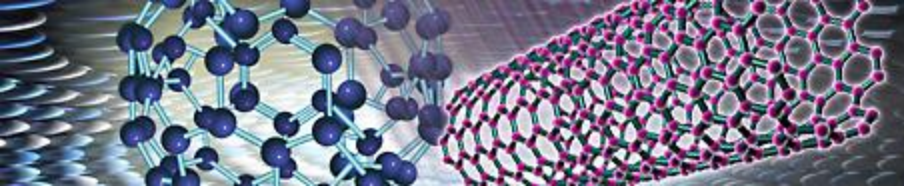
High Throughput Screening- multiple bioassays
Hierarchical Ranking- risk profiling based on physicochemical characteristics
Nano QSAR analysis- qualitative validation of predictions in vitro and in vivo



Predictive Modeling: PBPK



Particle toxicokinetics models- PNNL and Rutgers



NTP Nano EHS Efforts

- Carbon based fullerenes
  - Sub-chronic toxicity and immunotoxicity of C60-aggreates
  - Inhalation (50nm and 1um)- reports being written
  - Oral route- study initiated in 2012
- MWCNT
  - Sub-chronic inhalation toxicity and clearance (ongoing) on a select MWCNT
  - Chronic bioassay initiated early this year
- Nanosilver toxicity (IAA with NCTR)
  - Role of particle size on toxicity (testing 3 spherical sizes)
  - Oral and i.v. toxicokinetic & Sub-chronic toxicity studies ongoing

Nano EHS in CEBS

- Chemical Effects in Biological Systems database (CEBS) houses toxicological information of interest to health scientists.
- CEBS has a public and a private component.
- The public component houses over 9000 toxicological studies containing raw study data and metadata.
- Based within NTP at NIEHS
- Data from NTP Nano EHS and NCNHIR consortium efforts are being moved into CEBS and will be accessible to investigators/partners
  - Access to public as deemed fit

NIEHS
National Institute of Environmental Health Sciences

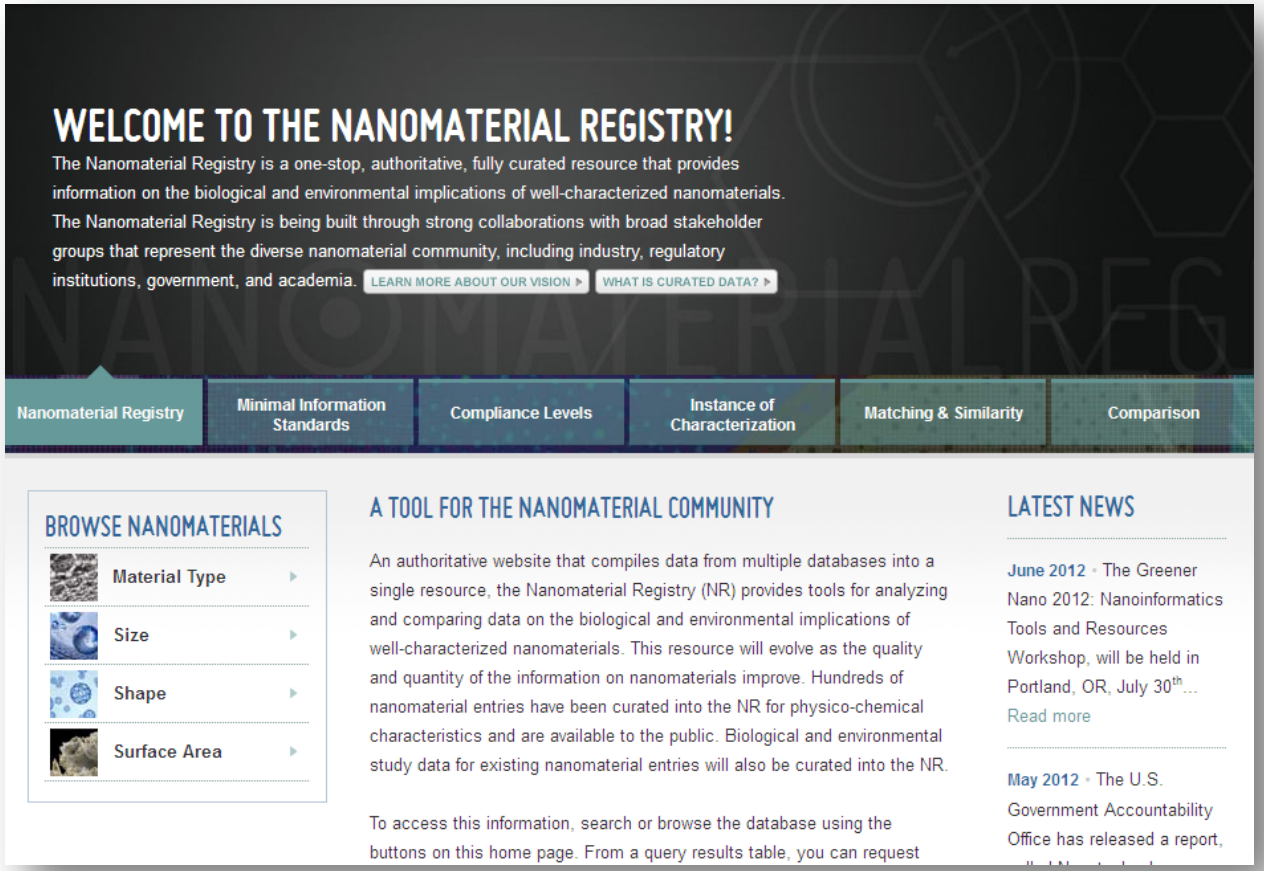
CEBS
Chemical Effects in Biological Systems

http://cebs.niehs.nih.gov

NANOMATERIALREGISTRY

Web Address:
www.nanomaterialregistry.org

A tool for the storing, sharing, and analysis of data from the nanomaterial community



Funded by:

NIEHS

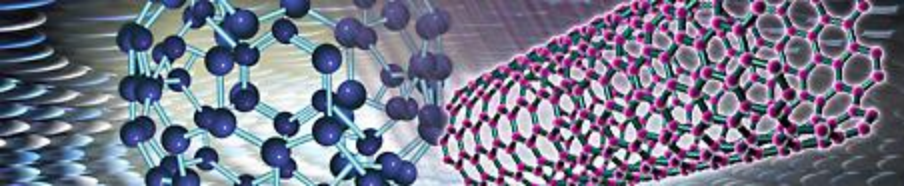
NIBIB

NATIONAL CANCER INSTITUTE

RTI INTERNATIONAL

Summary

- Success of Nano EHS research depends on coordinated, collaborative and integrated research efforts
  - Through multidisciplinary consortia
- NCNHIR consortium is open for collaboration
  - Investigators have access to ENMs, test systems and tools and collaborations
  - Chemical Effects in Biological Systems (CEBS) database
  - Hazard prediction and health effects assessment models
  - Next meeting of the NCNHIR will be held in September 2013.



Future Interests

- Continue our understanding on the influence of ENMs-PCPs on biological response-hazard characterization
  - Other routes of exposure (ingestion, dermal, etc.,)
  - Pristine ENMs to nanocomposites
  - Life cycle analysis
- Methods to quantify ENMs in diverse matrices including occupational and environmental exposures
- Support epidemiological studies integrated with personal monitoring

Thank You