NIEHS
National Institute of Environmental Health Sciences

NIEHS and NTP Activities Evaluating the Safety of Nanoscale Materials

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Washington DC, USA

U.S. Department of Health and Human Services
National Institute of Health
National Institute of Environmental Health Services

NIEHS and NTP

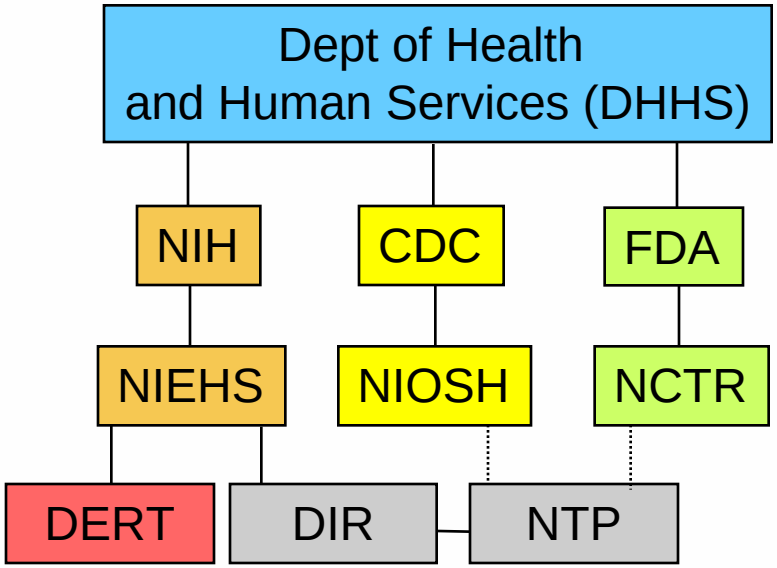
- National Institute of Environmental Health Sciences (NIEHS)
  - One of 27 research institutes and centers that comprise the National Institutes of Health (NIH)
  - Located in Research Triangle Park, North Carolina
  - Mission: to reduce the burden of human illness and disability by understanding how the environment influences the development and progression of human disease.
- National Toxicology Program (NTP) (ntp.niehs.nih.gov)
  - Interagency program headquartered at NIEHS, established in 1978 to coordinate toxicology testing programs within the federal government
  - Mission: to evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology.

Nanotechnology Environmental Health and Safety

- NIEHS identifies nanotechnology as a high priority
- Global demand for nanomaterials and devices will reach $3.1 trillion by 2015
- Unanticipated exposure
- Unknown health consequences
- Biological interactions at nano-scale (physical and chemical properties) are not known

Overview of NIEHS and NTP nano activities

- Funded by NIEHS
  - Division of Extramural Research and Training (DERT)
    - Grants
    - Training
    - Superfund
- NIEHS research
  - Division of Intramural Research (DIR)
    - Investigator Initiated research
    - Application of nanotechnology in EHS
  - National Toxicology Program (NTP)
    - Contract based research and testing



Nanotechnology-Environmental Health and Safety

- Nano Grand Opportunity (GO) program
  - Established a consortium to address critical research need: develop reliable and reproducible methods for toxicity evaluation of engineered nanomaterials (ENMs)
- NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR)
  - To gain fundamental understanding of nanomaterials interactions with biological systems
  - How diverse physical and chemical properties (PCPs) dictate these interactions
  - Translate observations from in vitro to in vivo
  - Aid development of risk assessment models to predict safety of ENMs based on PCPs

NIEHS Nano Grand Opportunity (GO) Program

- $13 Million from ARRA
  - Consortium-based research model to develop reliable and reproducible methods to test engineered nano material (ENM) toxicity
    - 10 NanoGO Grants
    - 3 Challenge Grants
- Ongoing studies to predict toxicity of 3 different well characterized ENMs
  - Human and rodent cell lines (4 assays)
  - Preliminary results from 8 labs doing round robin testing show high reproducibility of data

NIEHS Centers for Nanotechnology Health Implications research (NCNHIR)

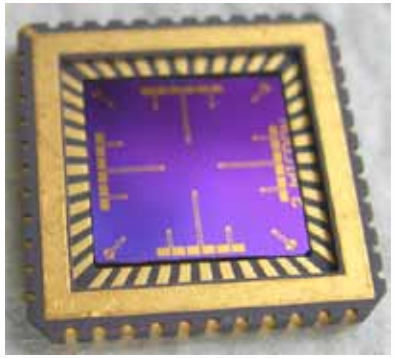
- Gain fundamental understanding of nanomaterial biological interactions as dictated by their physical and chemical properties
  - Five U19 Grants
  - Three U01 Grants
- Contract Support:
  - National Characterization Laboratory (NCL)
  - NIBIB: Nano Registry

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

NIBIB
NATIONAL INSTITUTE OF BIOMEDICAL IMAGING AND BIOENGINEERING

Nanotechnology Applications

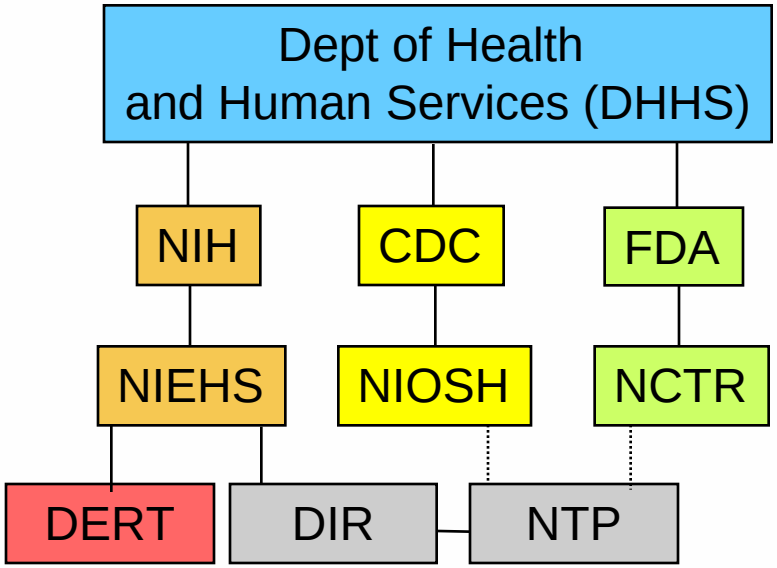
- Nanotechnology-based sensors
  - Characterization of exposures to multiple analytes with high sensitivity and temporal resolution in small inexpensive devices.
- Bioremediation Using Nanomaterials
  - Nano-towel to capture mercury vapor
  - Nano-scaled iron particles for water cleanup
  - Nanoparticles applied to reduce PCBs,
  - PAHs, PBDEs



Superfund Research Program

Nano at NIEHS

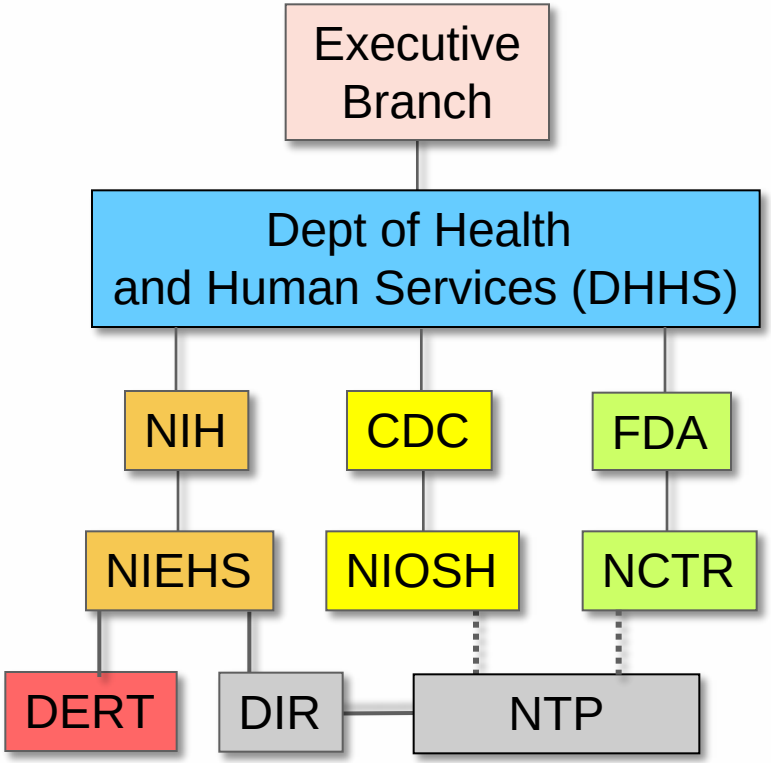
- Funded by NIEHS
  - Division of Extramural Research and Training (DERT)
    - Grants
    - Training
    - Superfund
- Research at NIEHS
  - National Toxicology Program (NTP)
    - Contract based research and testing
  - Division of Intramural Research (DIR)
    - Investigator Initiated research
    - Application of nanotechnology in EHS



National Toxicology Program?

- Interagency program
  - Established in 1978 to coordinate toxicology research in DHHS
  - Headquartered at NIEHS, part of NIH
- Research on submitted “nominations”
  - Thousands of agents evaluated in comprehensive toxicology studies
  - GLP compliant “testing” through government contracts
- Analysis activities
  - Report on Carcinogens
  - Center for the Evaluation of Risks to Human Reproduction (CERHR)
  - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

ntp.niehs.nih.gov



NTP Research and Testing Programs

- AIDS therapeutics
- Air/Food/Water contaminants
- Cardiovascular disease/toxicity
- Dietary supplements
- DNA-based therapeutics
- Endocrine disruptors
- Flame retardants
- Green chemistry
- Herbal medicines
- Mold
- **Nanoscale materials**
- Occupational exposures
- Phototoxicology
- Radiofrequency radiation
- Risk assessment issues/mixtures

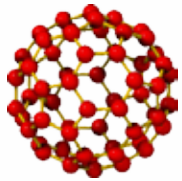






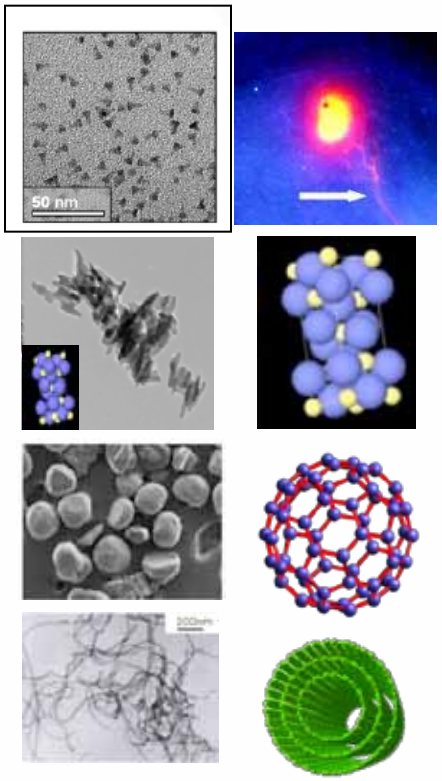




NTP Activities on nanomaterial classes

- Cadmium based “quantum dots”
  - Role of skin integrity on pharmacokinetic studies after dermal exposure
- Titanium dioxides
  - Dermal pharmacokinetics
- Carbon based fullerenes; Pulmonary and oral toxicity
  - Impact of size of C60 aggregates
- Multiwalled carbon nanotubes
  - Influence of length and diameter on pulmonary toxicity
- Exposure assessments
- Nanosilvers
  - Role of particle size and shape on PK and toxicity



Lack of dermal Penetration of skin by Quantum dots

- Supported by Interagency agreement between NIEHS and NCTR/FDA
- Gopee et al 2007, 2009
- Dermal exposure to SKH-1 mice
  - Intact and dermabraded
- Use of quantum dots as model system
  - ((CdSe)CdS)-PEG (Emax621nm)
  - Quantitation of Cd in tissues by ICP-MS and confocal fluorescence microscopy
- No consistent penetration through intact skin

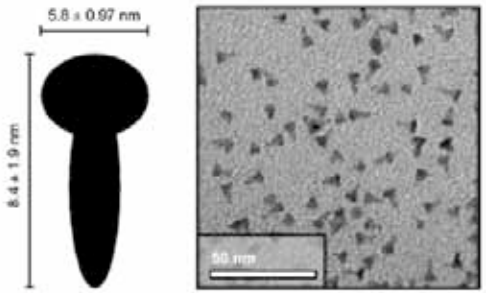


FIG. 1. Characterization of QD using TEM. The QD were characterized using TEM, and the average QD CdSe core size was nail shaped with an 8.4 ± 1.92 nm length and 5.8 ± 0.97 nm width, resulting in an aspect ratio of 1.45.

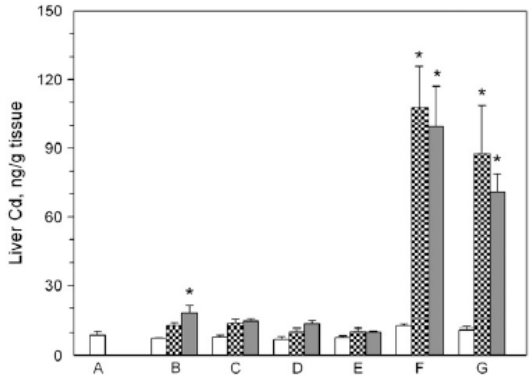


FIG. 4. Cd levels in the liver of SKH-1 mice topically applied with CdSe QD suspended at 9μM in an oil-in-water emulsion. The liver was removed from mice ($n = 3$ per group) topically treated as follows: (A) no QD applied; (B) QD applied to normal skin; (C) QD applied to tape-stripped skin; (D) QD applied to acetone treated skin; (E) QD applied to untreated skin and covered with occlusion patch; (F) QD applied to dermabraded skin; (G) QD applied to dermabraded skin and covered with occlusion patch. The animals were sacrificed at 0 h (open bar), 24 h (hatched bar), or 48 h (filled bar) after application of the emulsion.

- Gopee et al 2007, 2009

Lack of dermal penetration by metal oxides

- Gopee et al 2009
- Dermal exposure to SKH-1 mice
  - Intact and dermabraded
- Use of uncoated anatase P25 Ti02
  - 5% Ti02 in representative emulsion
  - Spatial evaluation by Cytoviva dual mode darkfield microscopy
  - Quantitation of Ti in sentinel tissues by ICP-MS
- No consistent penetration through intact or dermabraded skin

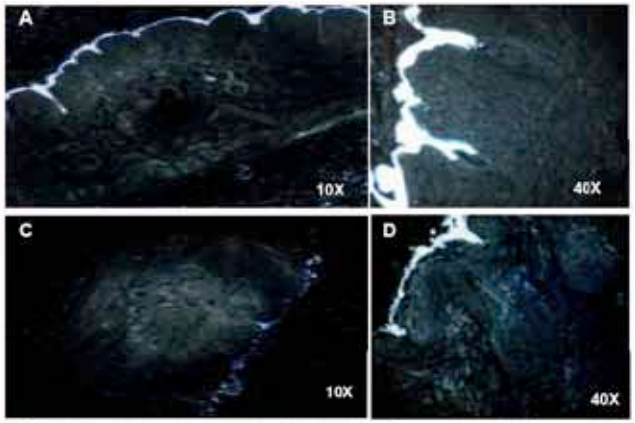
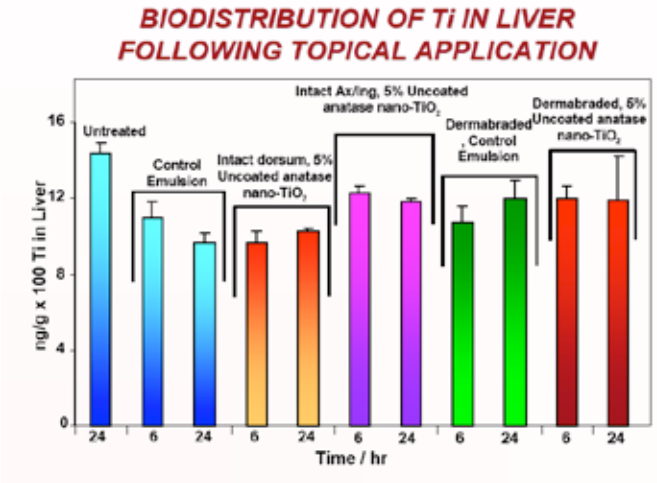
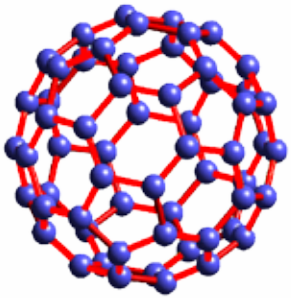


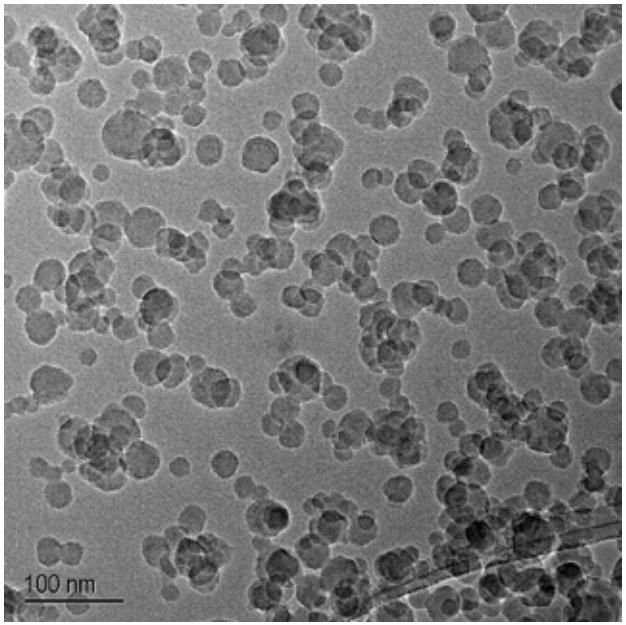
Figure 3. Topically applied TiO_2 was visualized on the surface and in hair follicles with no apparent penetration beyond the stratum corneum in dermabraded (A & B) and intact (C & D) skin.

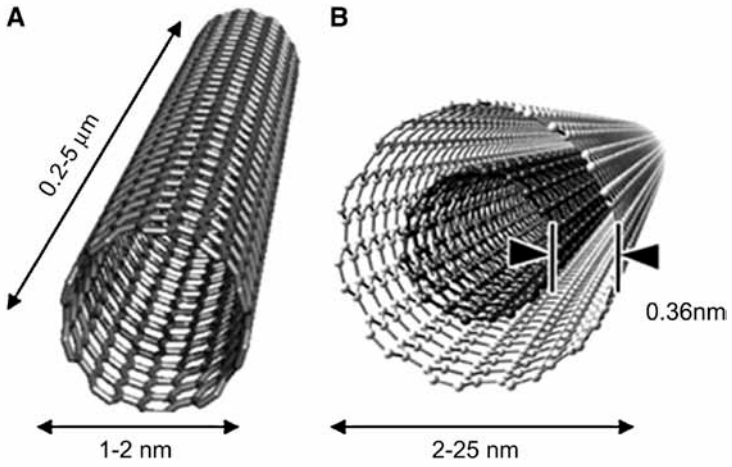


Carbon-based NSMs

- Fullerenes
  - eg C60 “Buckyballs”
- Nanotubes”
  - Single walled (SWNT)
  - Multi walled (MWNT)
- Nanofibres/nanofibrils



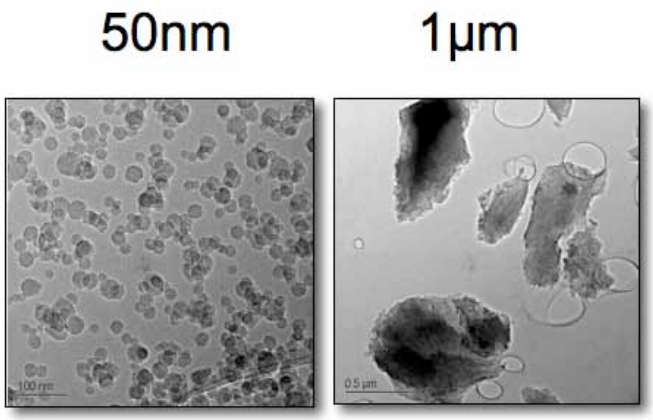




Source: J Nucl Med 48: 1039

Pulmonary toxicity evaluation of Fullerene-C60

- NTP inhalation study conducted under GLP
  - 90 days-nose only exposure, 3hrs/day, 5d/wk
  - B6C3F1 mice and Wistar-Han rats
  - 50nm (0.5 and 2 mg/m^3)
  - 1um (2, 15 and 30 mg/m^3)
- Preliminary findings
  - Shorter clearance in mouse vs rat
    - Not size effect
  - No biologically significant toxic responses
  - Expected response to particles
  - Comparable surface area-based doses between 50nm and 1um study



Particle size	MMAD*	Count Median Diameter (CMD)	Surface area (SA) m^2/g	Concentrations mg/m^3	SA adjusted concentrations m^2/m^3
50nm	76nm	36nm	67	0.5	0.034
	88nm	44nm	56	2	0.112
1um	1.15µm	--	5.4	2	0.016
	1.14µm	--	5.8	15	0.087
	1.19µm	--	5.5	30	0.165

* Mass median aerodynamic diameter (MMAD) calculated from CMD using Hatch-Choate relationships assuming log-normal aerosol size distribution then converted to MMAD assuming a particle density of 1.72 g/cc
For brevity particle size details are shown for the rat studies. Comparable data were seen for the mouse studies

Occupational exposures to Carbon nanomaterials

- Interagency agreement with NIOSH
  - Chuck Geraci and Mary Schubauer-Berigan, NIOSH
- Evaluate the feasibility of industry-wide exposure assessment and epidemiology studies of US manufacturers of engineered carbonaceous nanomaterials (ECN)
- Information includes
  - Nanomaterials produced, characterization of size and shape
  - Size of worker population by facility
  - Tonnage of materials used or produced
  - Workforce size
- Status
  - Approx 60 companies, >50% CNTs, fullerenes the next most common
  - Several hundred workers, workforce growing annually

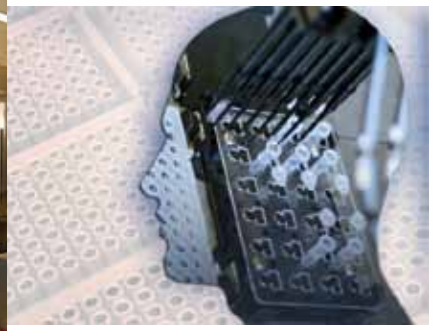
A National Toxicology Program for the 21st Century

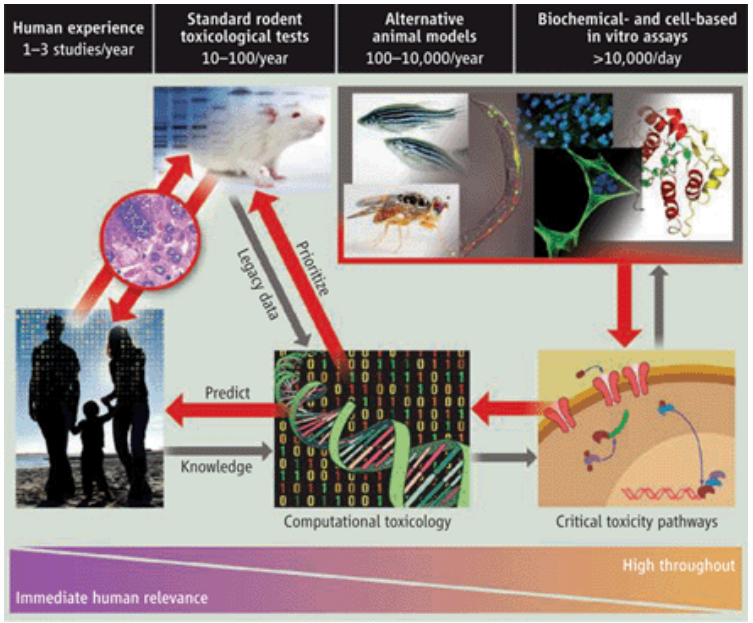
- Roadmap to Achieve the NTP Vision (2004)
  - "To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target specific, mechanism-based, biological observations."

- Key activities
  - Develop high-throughput capabilities
  - Further evaluate and refine the use of non-mammalian models
  - Improve the use of toxicokinetic information

NTP High Throughput Screening

- Main goals
  - Identify mechanisms of action
  - Prioritize substances for further in-depth toxicological evaluation
  - Develop predictive models for in vivo biological response
- Tox21
  - Collaboration with USEPA, NCGC
  - Focus on small molecules
  - EPA testing set includes nanomaterials





Nanotechnology at NIEHS

- NIEHS is actively involved in US federal research efforts coordinated by National Nanotechnology Initiative
- Took leadership roles in addressing environmental health and safety concerns of widespread use of nanotechnology.
- Supports research programs in:
  - Health implications of nanotechnology based materials and devices
  - Application of nanotechnology-based sensors/tools for environmental monitoring
  - Superfund remediation efforts

Key Personnel

- NIEHS; Grants Programs
  - EHS - Sri Nadadur, PhD
  - Superfund: Heather Henry, PhD
  - Applications-David Balshaw, PhD
- NIEHS; Toxicology Liaison (Bethesda office)
  - Chris Weis, PhD
- National Toxicology Program
  - NIEHS; Nigel Walker, PhD
  - FDA; Paul Howard, PhD
  - NIOSH; Mark Torasson PhD, Charles Geraci PhD, CIH