Battelle

The Business of Innovation

Advancing Hazard Assessment and Dosimetry for Nanomaterial Risk Assessment

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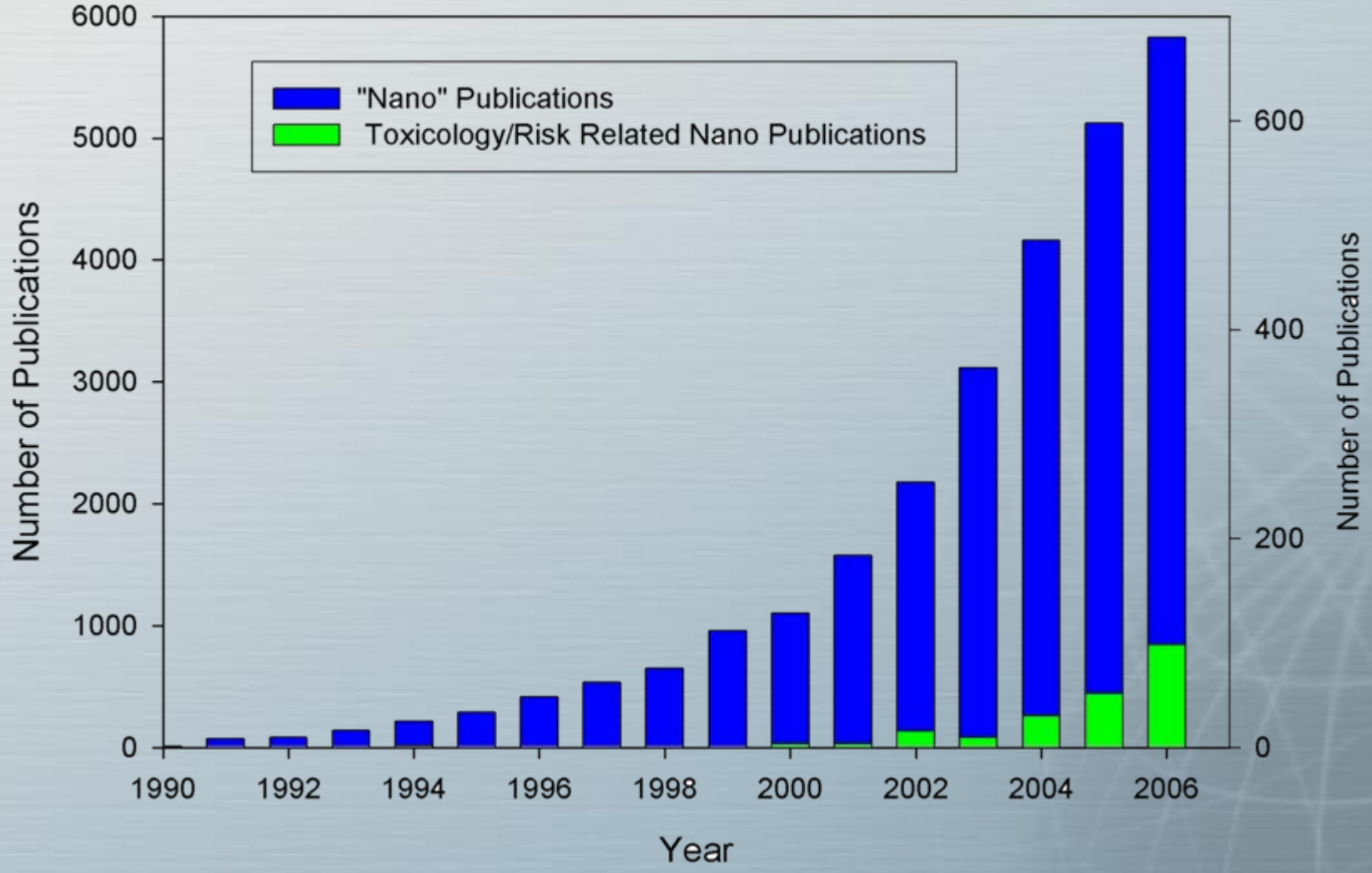
Pacific Northwest National Laboratory

Unknown

Nanomaterial Human Health Risks and Risk Assessment

Insufficient Data

Nanotoxicology is in its Infancy



Nanotoxicology is in its Infancy

Do the unique material properties of nanomaterials equate to unique biological properties as well?

Which of these properties are responsible for the biological effects?
Are the effects limited by the traditional defintion of nanomaterial (1-100 nm)?

Who should be studying the biocompatibility or toxicity of these new materials?

What studies should be done?

Where do we obtain high quality materials for testing?

Nanomaterial Risk & Safety Assessment

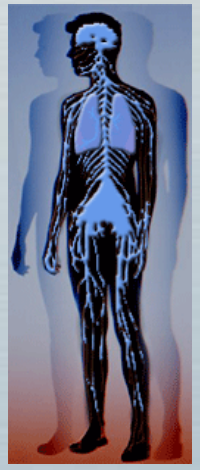
Exposure Assessment

Exposure Characterization

P. Chemistry Aggregation

Particokinetics

Respiratory Dosimetry



Systemic Disposition

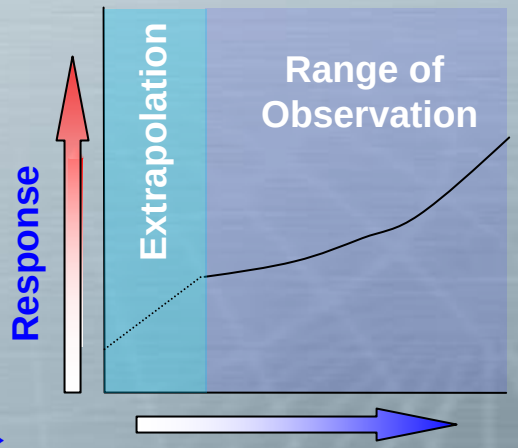
Hazard Assessment

Particodynamics

Molecular→Cellular → Tissue → Organism

Dose-Response

Dose Response and Risk Assessment



Risk Characterization

Nanomaterial Risk & Safety Assessment

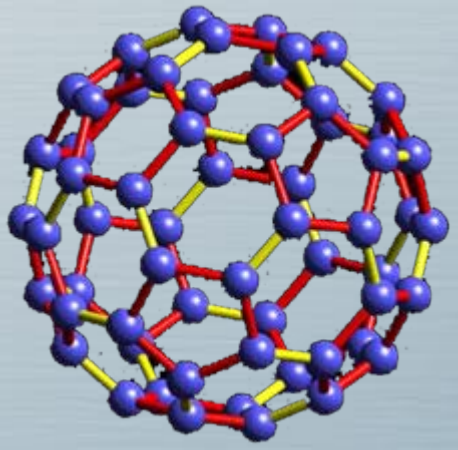
What we need to know:

- Extent and duration of exposure—***DOSE***
- Characteristics of the material—***DOSE***
- Adverse effect—***RESPONSE***
- How rodent dose-response relates to human dose-response—***EXTRAPOLATION***
- How the toxicity of one well studied material can be related to untested materials

Outline

- Hazard Assessment
  - Major challenges
  - Integrated research program for determing mode of action and physicochemical drivers of toxicity
  - NIOSH collaboration on the hazard assessment of multiwalled carbon nanotubes.
- Dose-Response Assessment
  - Major Challenges
  - Dosimetry for In vitro studies and extrapolation
  - Dosimetry for In vivo studies and extrapolation

Hazard Assessment



Major Challenges in NM Hazard Assessment

- Resource Limitations
  - The multitude of materials to test
  - The cost of individual studies
- Exposure generation and characterization
  - Exposures difficult to engineer and characterize
  - The availability of reference and verified test materials
- Properties influencing toxicity are unknown
  - No standard approaches for testing
  - Too many test systems
  - Unknown mode(s) of action
- Absence of benchmark toxicity data

Too Many Test Systems?

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Available online at www.sciencedirect.com

ScienceDirect

Toxicology Letters 168 (2007) 176–185

Toxicology Letters

www.elsevier.com/locate/toxlet

Mouse **Oral**

Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration

Jiangxue Wang[a,b], Guoqiang Zhou[a,b]
Tiancheng Wang[c], Yongmei Ma[d,*,1], Gu
Yufeng Li[a,b], Fang Jiao[a,b], Yi

Comparative toxicity of nano-scale TiO_2, SiO_2 and ZnO water suspensions

IV

Nano-Sized Carbon Blacl
Atherosclerosis in LDL-R

Yasuharu Niwa, PhD; Yumiko Hiura,
Masayuki Yokode, MD

Instillation

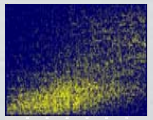
Comparative Pulmonary Toxicity Assessments of C_{60} Water Suspensions in Rats: Few Differences in Fullerene Toxicity in Vivo in Contrast to in Vitro Profiles **Rat**

Christie M. Sayes,† Alexander A. Marchione,‡ Kenneth L. Reed,† and David B. Warheit*,†

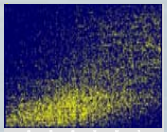
Hazard Assessment Needs Comprehensive, Unbiased, Integrated Assessments

Microarrays

Proteomics

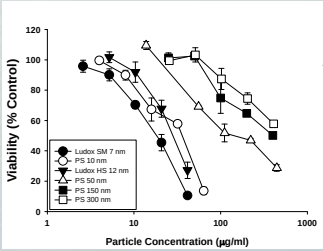


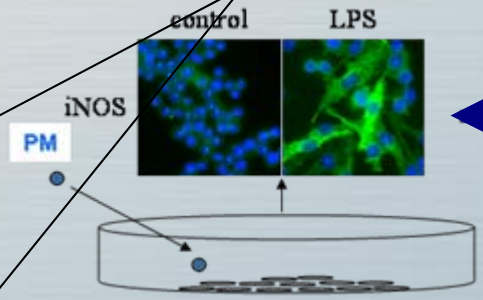
Proteomics



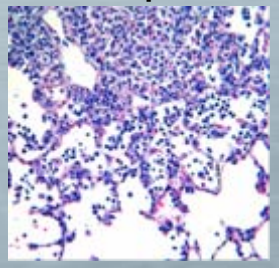
Microarrays

Conventional Tox Endpoints



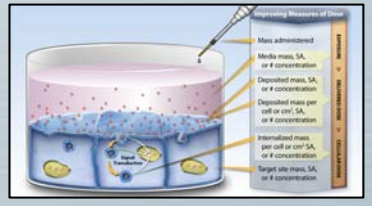


Conventional Tox Endpoints

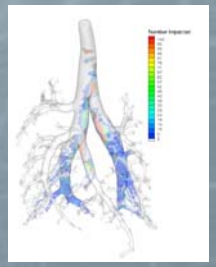


In Vitro ⟷ **In Vivo**

Dosimetry



Dosimetry



MOA
QSAR
Toxicity
Dose-Response

⟷

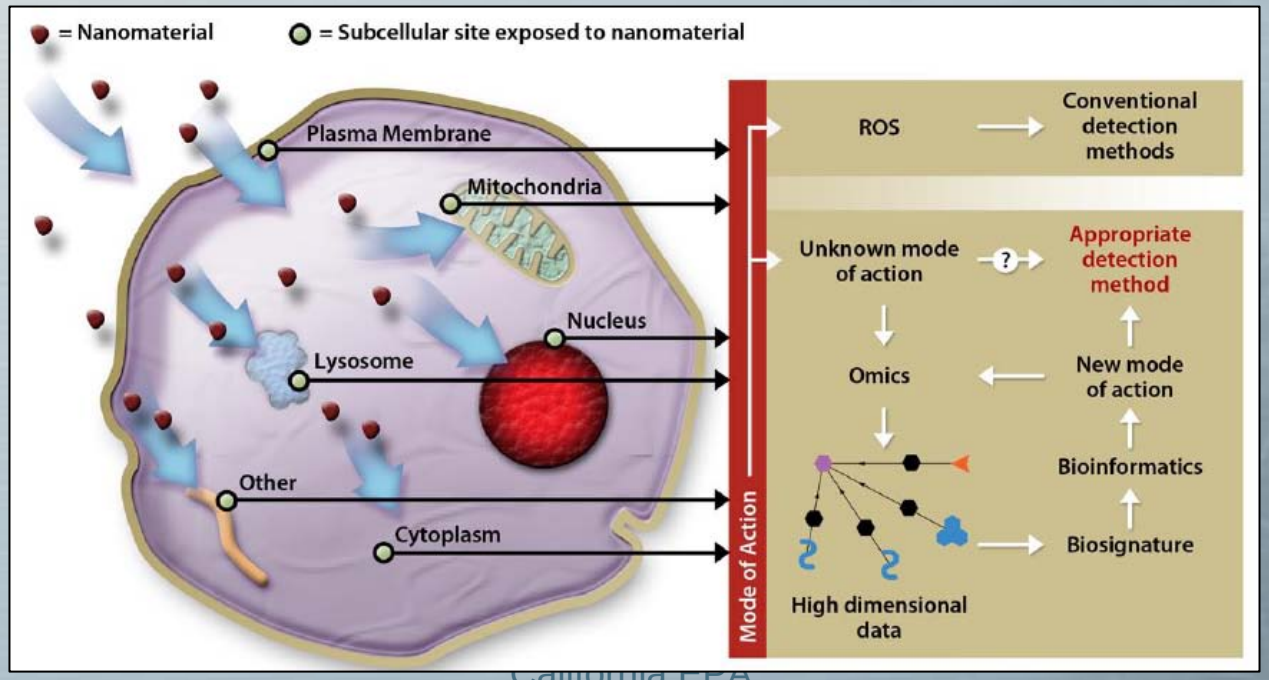
MOA
QSAR
Toxicity
Dose-Response

What Information do Proteomics and Genomics Provide?

Stimulus → **DNA** → **mRNA** → **Proteins** → **Change in Function**

Genomics: Global assay of cellular response based on the transcriptome

Proteomics: Global assay of cellular response based on the proteome



Hazard Assessment Part I: In Vitro

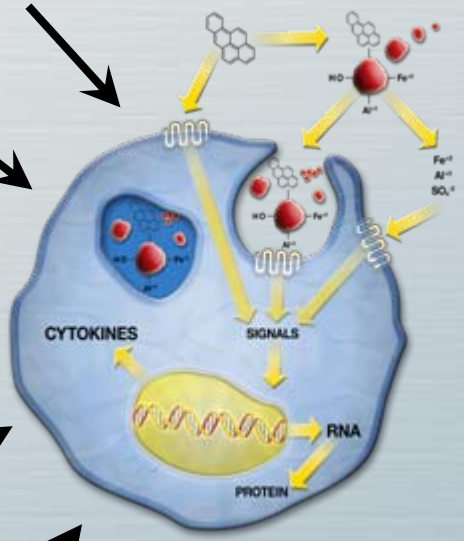
Amorphous Silica

LPS

Minusil

Carbon Nanotubes

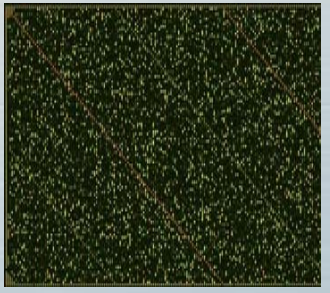
Urban Particulate



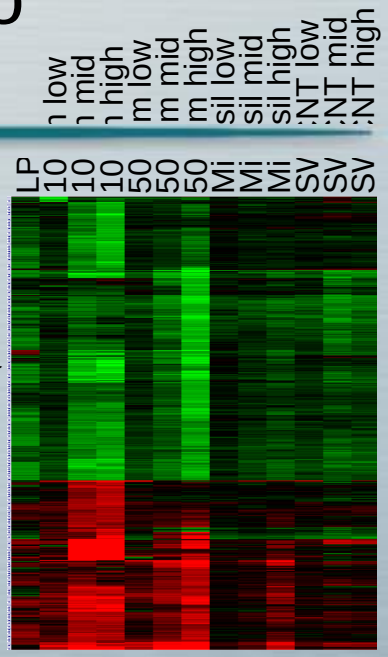
RAW 264.7 Macrophage

Conventional Tox Endpoints

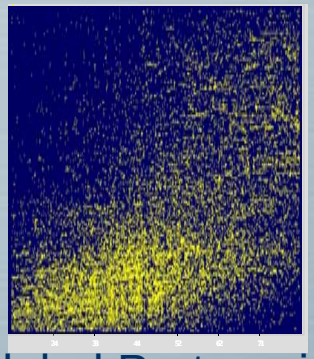
2hr



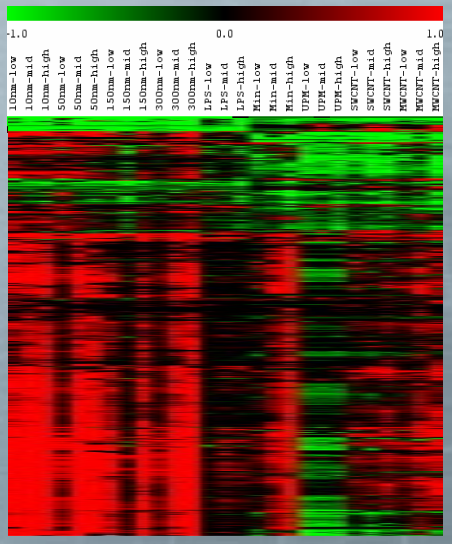
Microarray



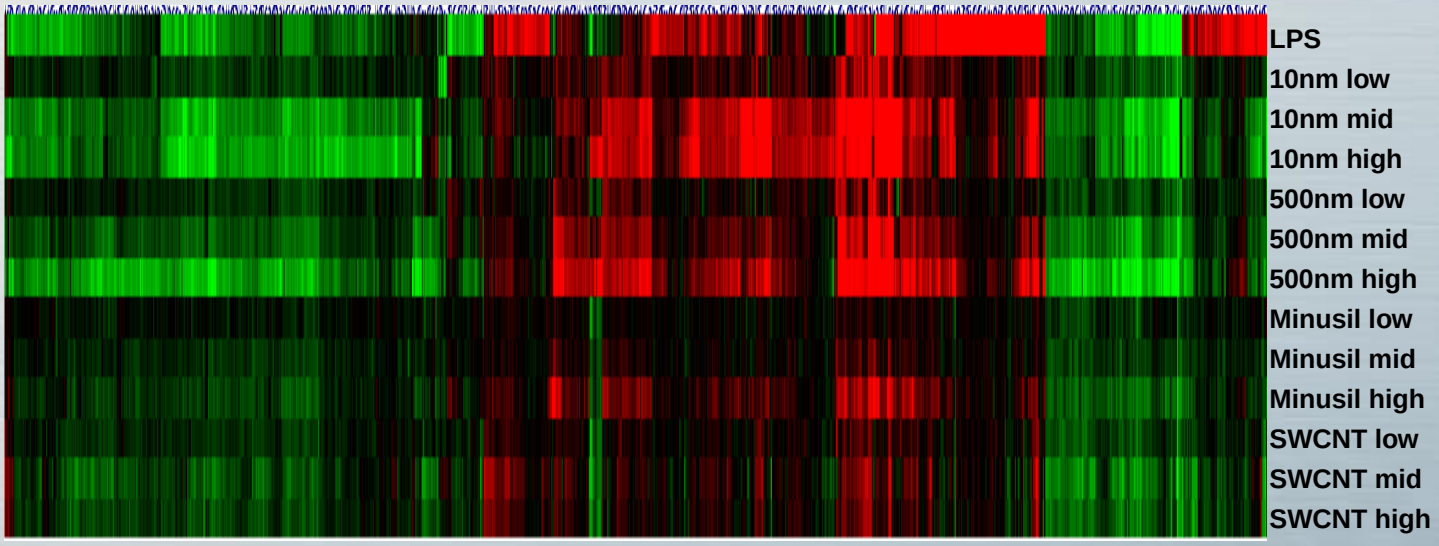
24hr



Global Proteomics (Secretome)

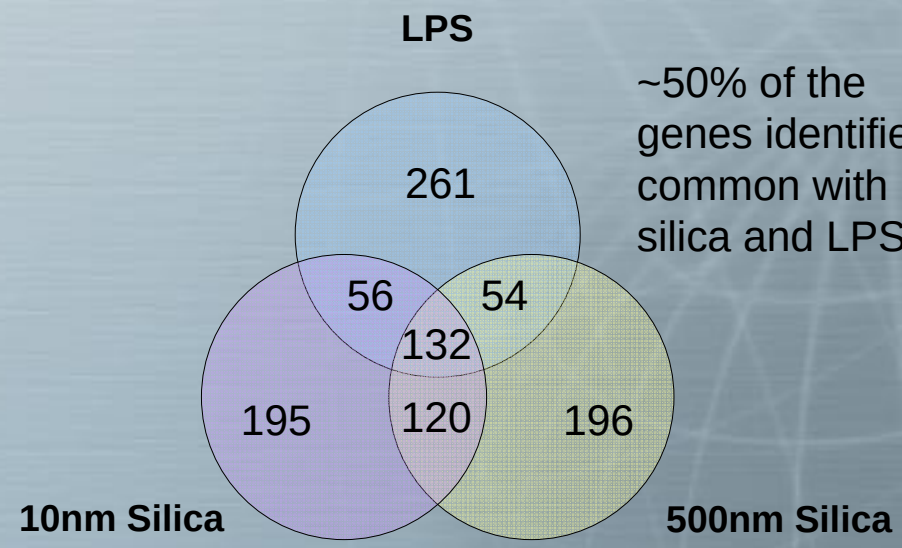


Comparison of Responses Between Stimuli (2hr exposure)



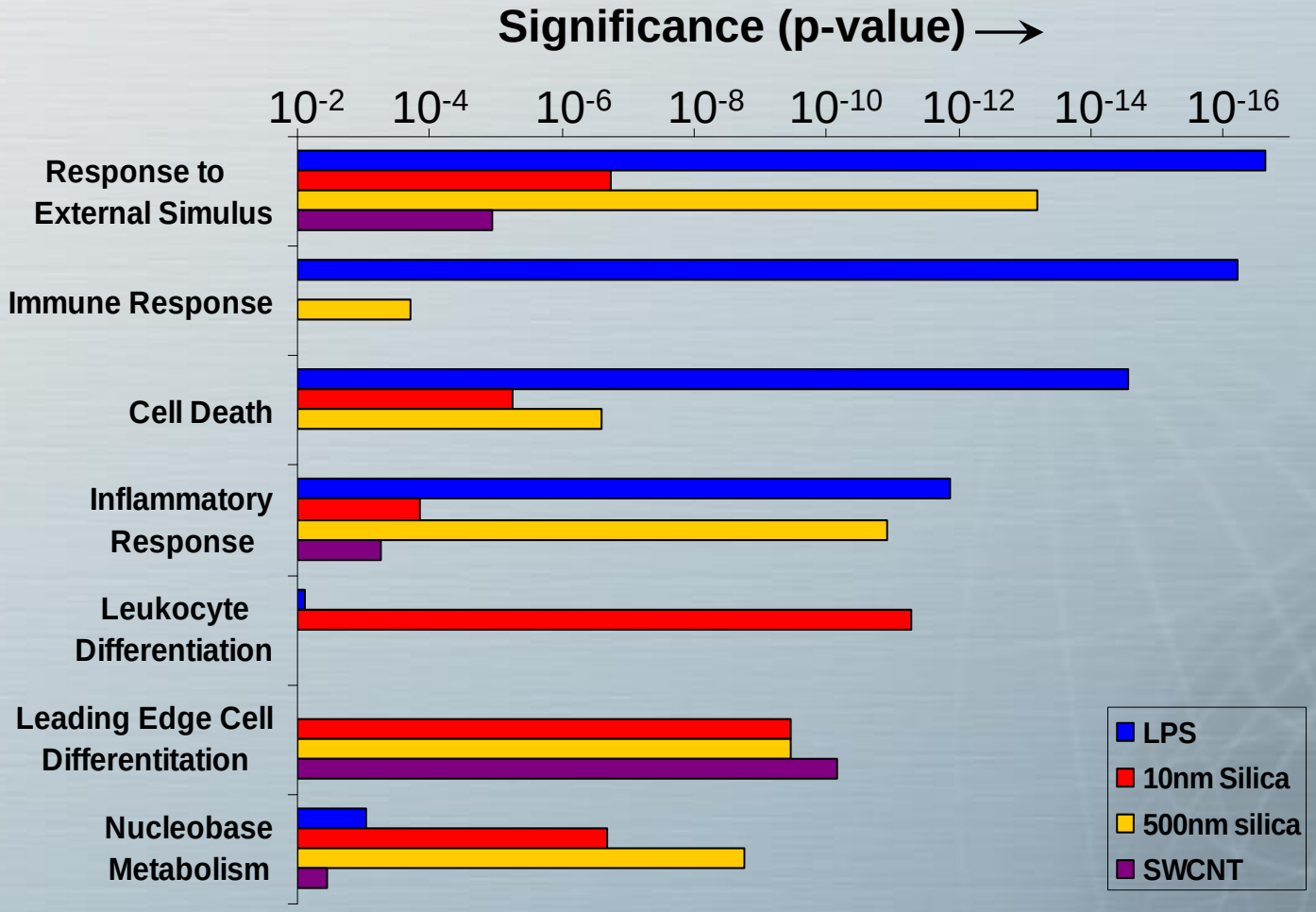
Treatment	# Genes
LPS	503
10nm Amor silica	503
500nm Amor silica	502
Minusil	56
SWCNT	67

Total (union of all): 1037 genes

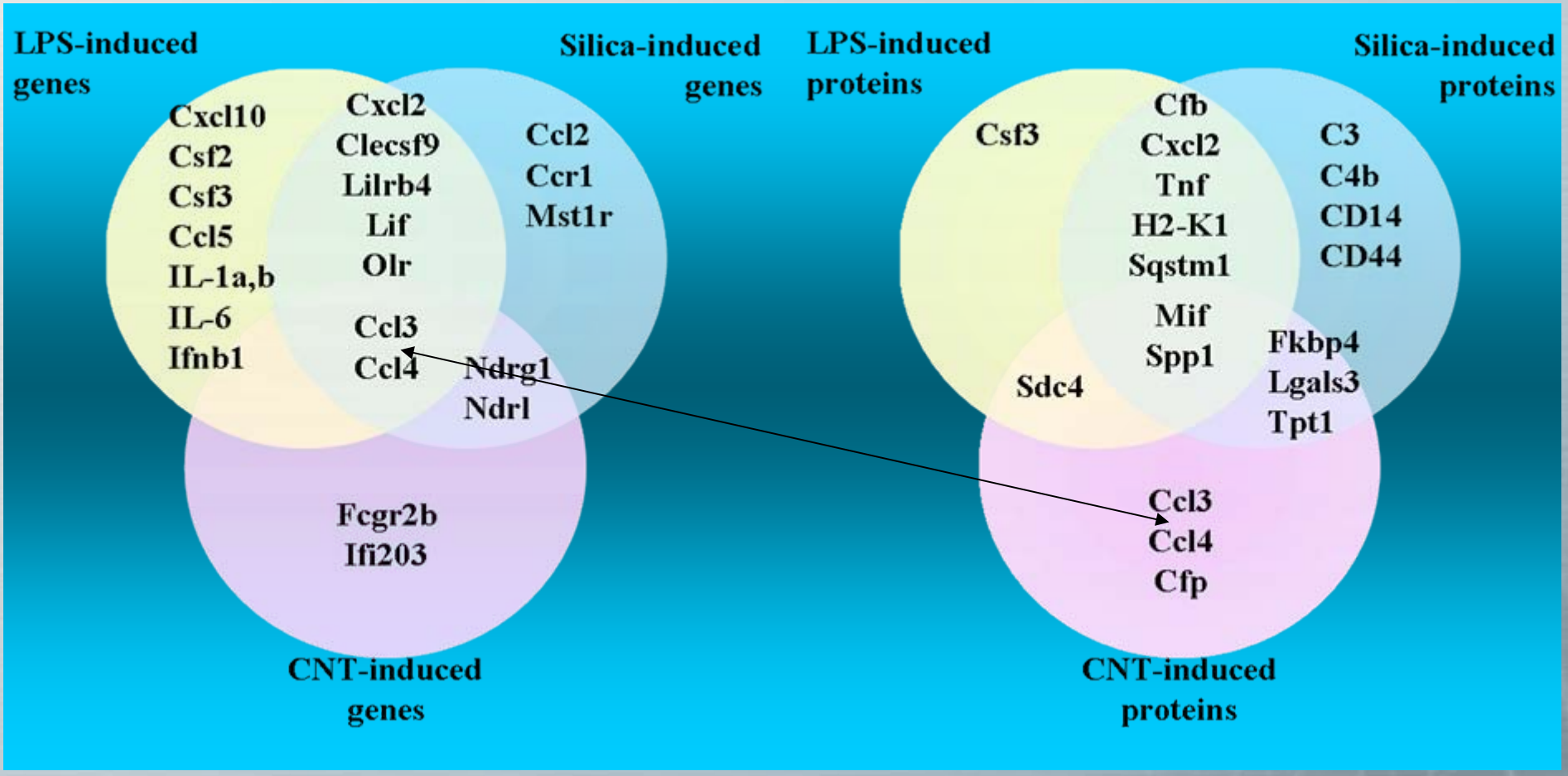


~50% of the genes identified common with silica and LPS

Functional Gene Set Enrichment Analysis: Major Cell Processes



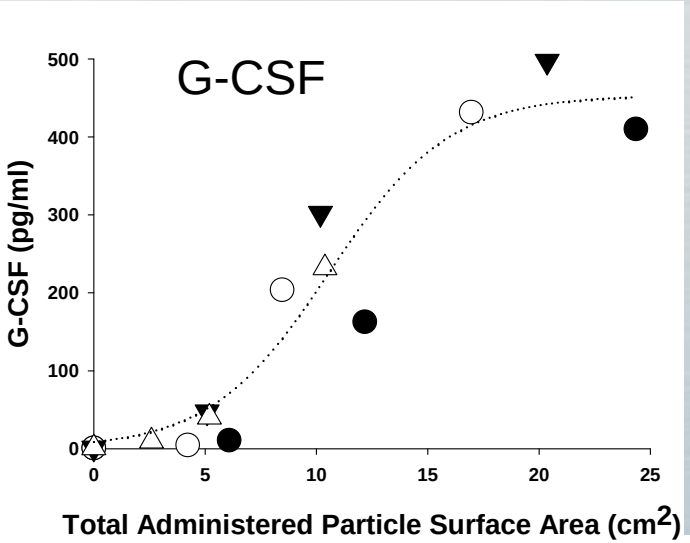
Inflammation-Related Biomarkers Identified by Integrated Proteomic and Genomic Analyses

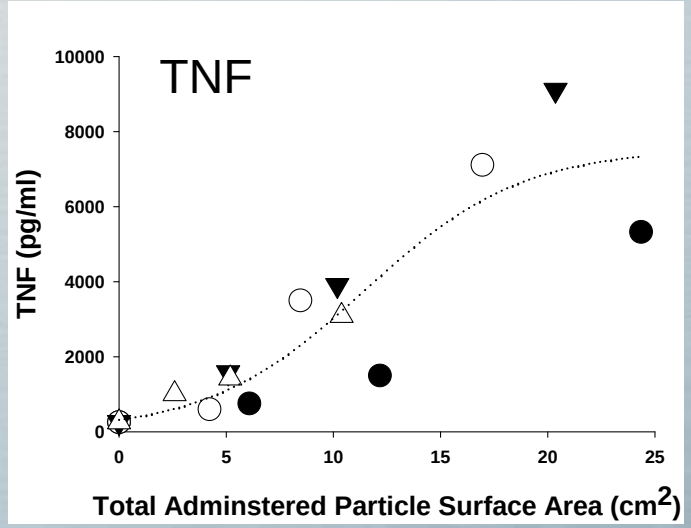


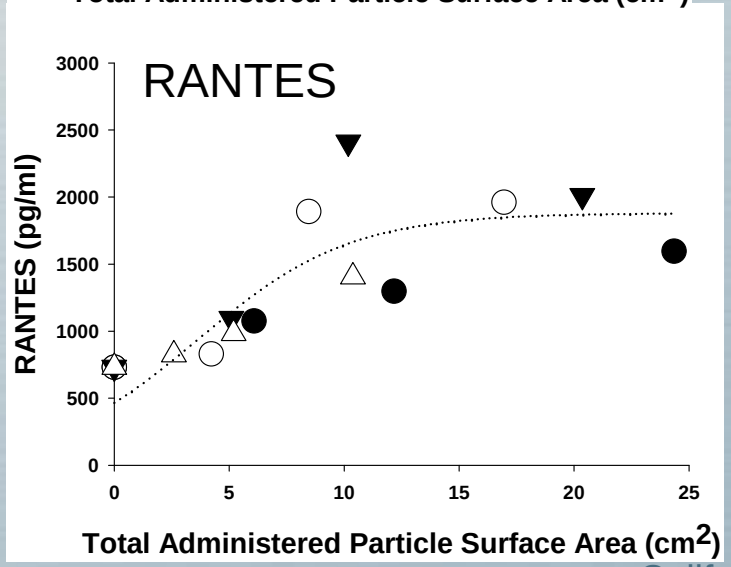
The pattern of protein expression and secretion can distinguish between different macrophage-activating stimuli.

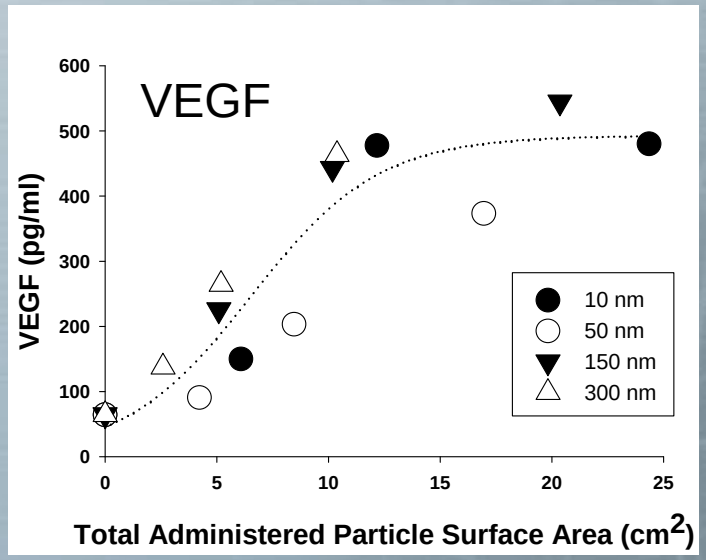
Exposure-Response Relationship for Secreted Protein Pathway Markers

(Protein Microarray ELISA Results, R. Zangar)

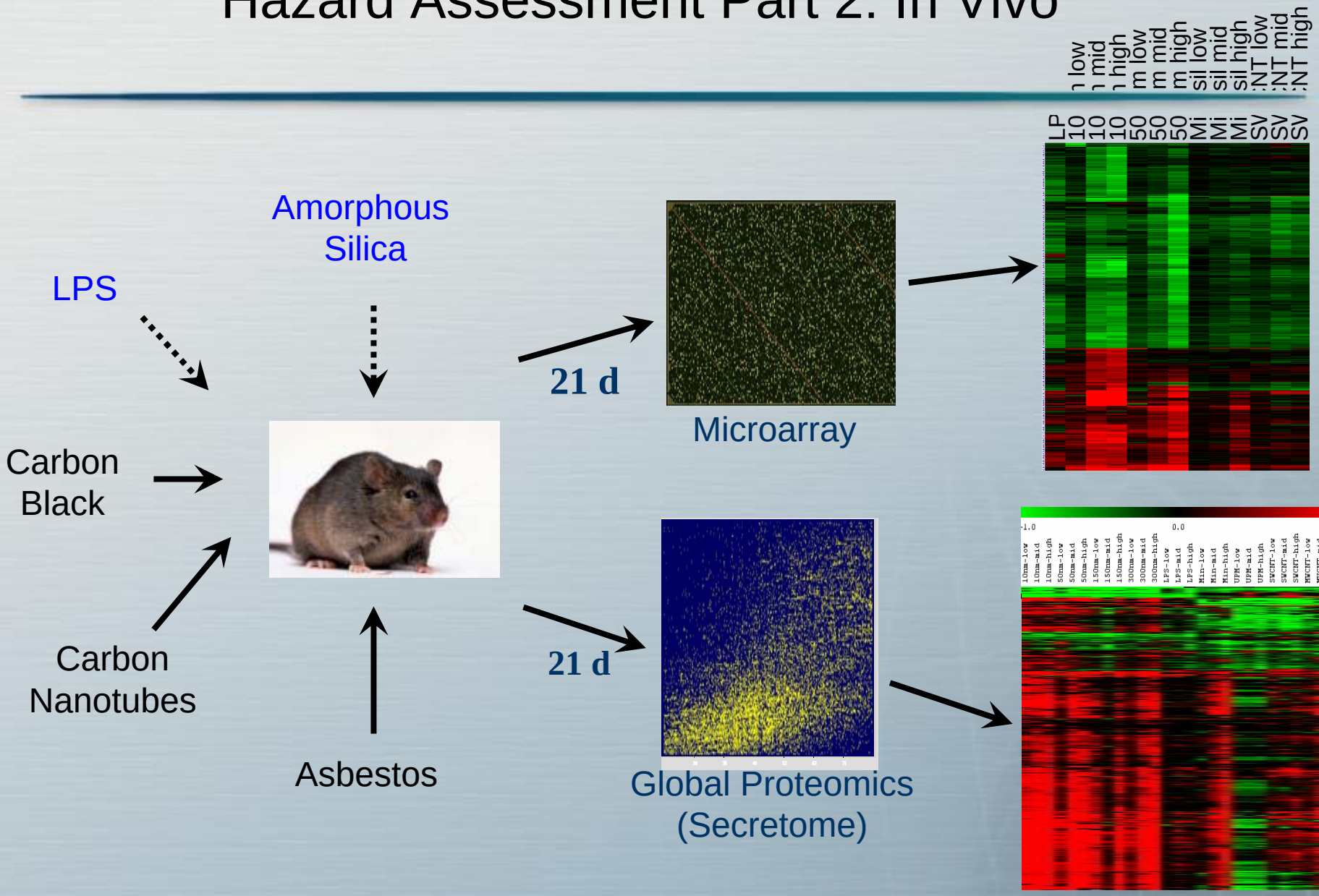






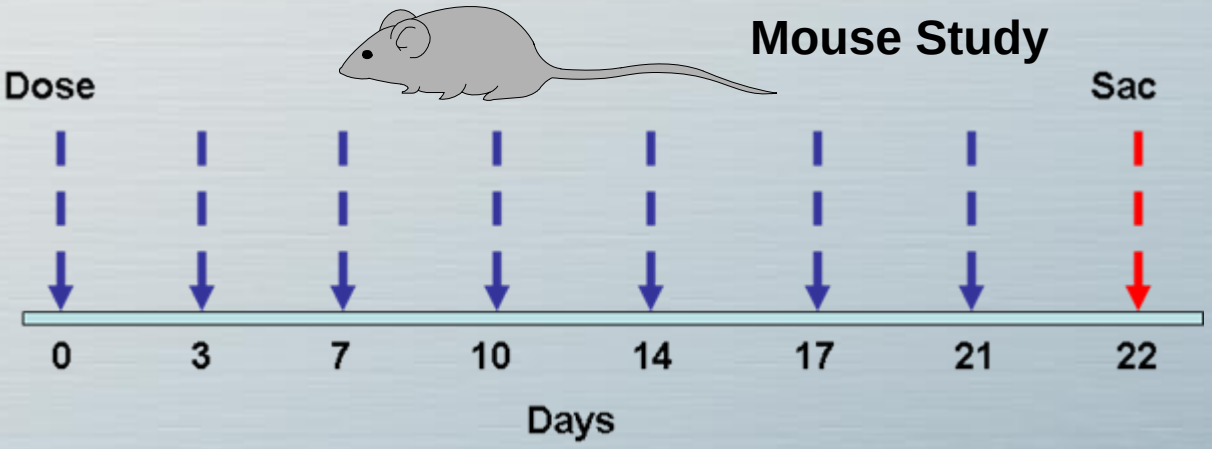


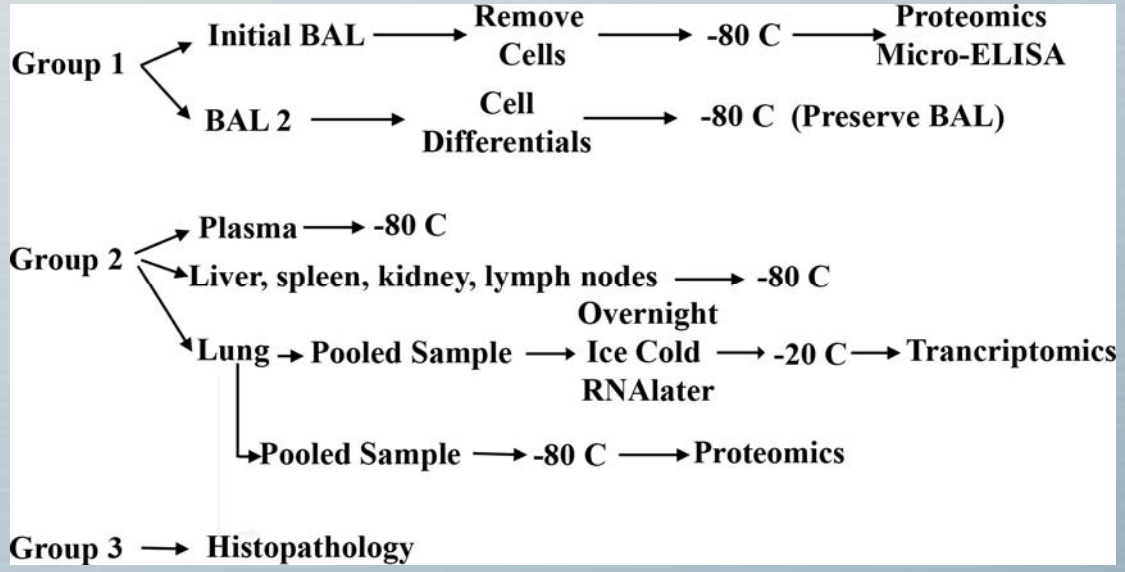
Hazard Assessment Part 2: In Vivo



NIOSH Collaboration

SWCNT
Carbon Black
Asbestos





Comparative Toxicology of SWCNT & Asbestos

↓

Proteomics
Genomics
Cytokines
Histopathology

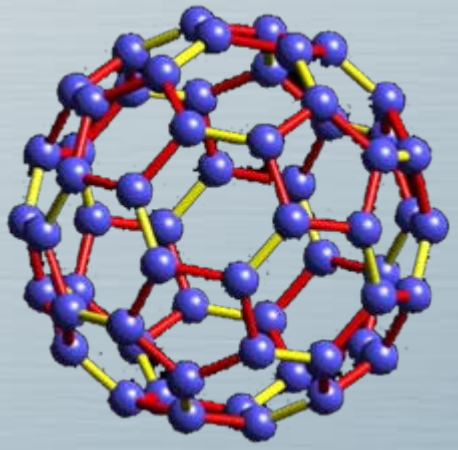
↓

Hazard Relative to Asbestos

What Does the Integrated Approach Provide Us?

- An improved basis for understanding the hazards posed by nanomaterials:
  - Mode of action
  - Biomarkers of response for high throughput assays
  - Comparative toxicology
  - A basis for determining the predictive power of in vitro studies
  - Comparison of short-term responses for new materials (SWCNT) to well studied materials (Asbestos)
  - Fundamental NM properties driving toxicity

Dose Response Assessment

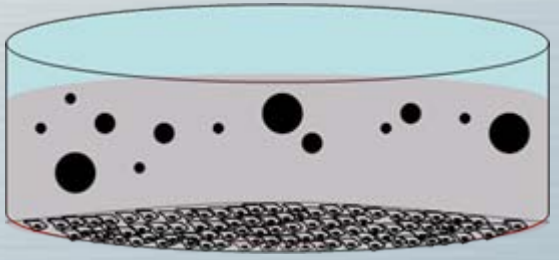


Major Challenges for Dose-Response Assessment

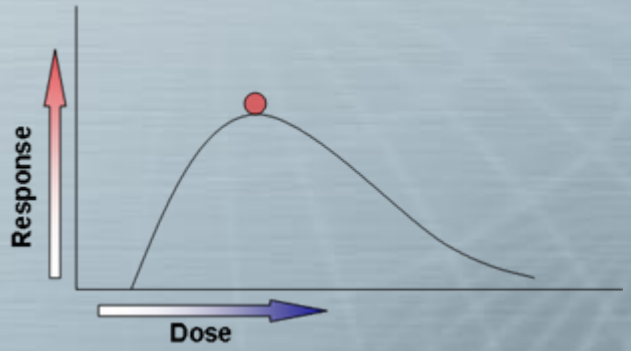
- What is dose?
  - Size, shape, particle number, agglomeration state, surface chemistry, reactivity, surface area? Dose rate is also important.
  - Do researches have all the tools necessary to measure these characteristics?
- What is dose for in vitro studies?
  - 67% of published NM toxicity studies use in vitro systems.
  - Mass concentrations are typically reported.
  - Cannot be compared to doses for in vivo studies.
  - How do you measure NM characteristics in liquid systems?
- How do you extrapolate animal study doses to human doses
  - This extrapolation is required for all risk assessments based on animal studies

Cell Culture is a Standard Tool for Toxicity Testing

- Toxicity testing of nanomaterials will involve in vitro testing
- The paradigm for chemicals is being used without consideration of the unique kinetic differences between chemicals and particles in solution:
  - Response is considered a function of the nominal media mass or surface area concentration (cm^2/ml, µg/ml)



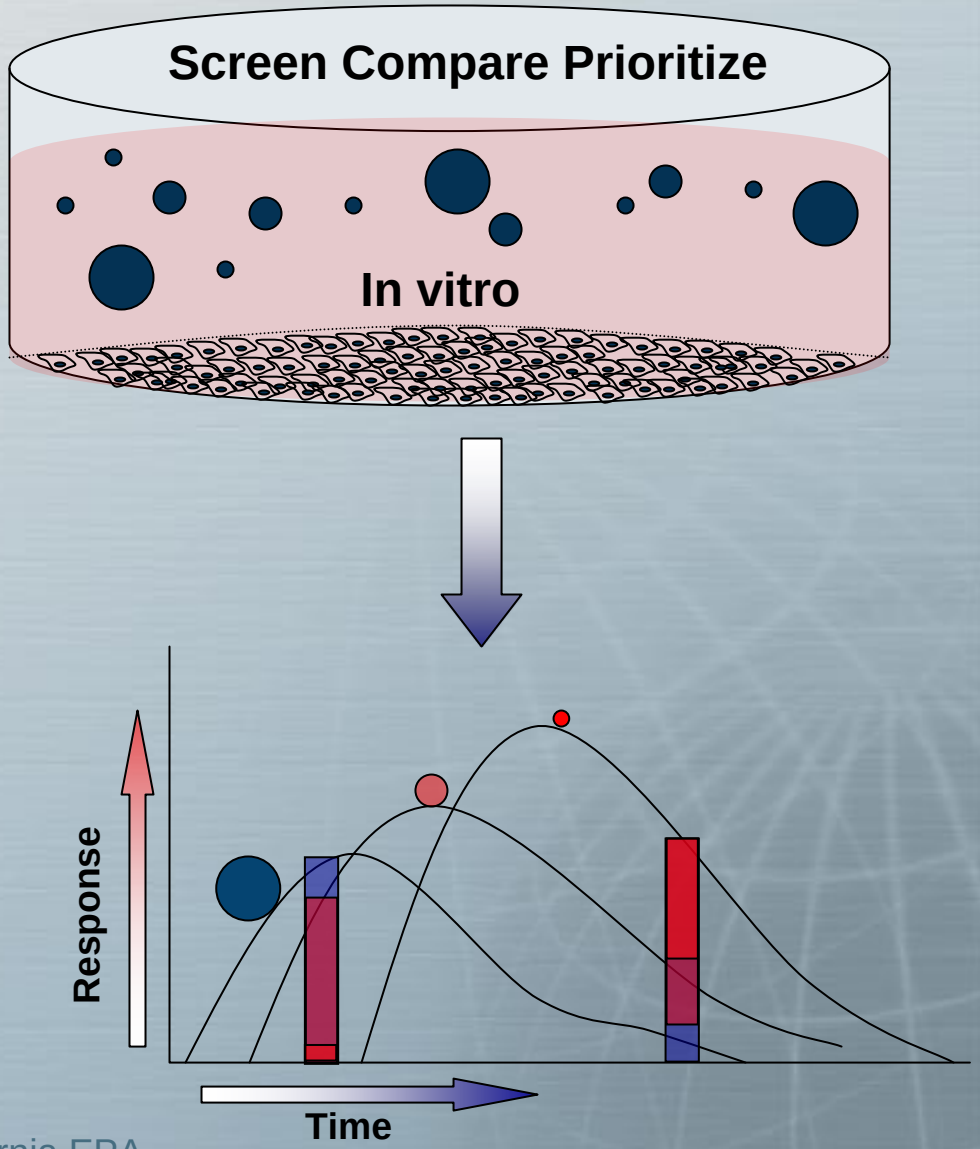
Expose cells to selected concentrations of suspended particles



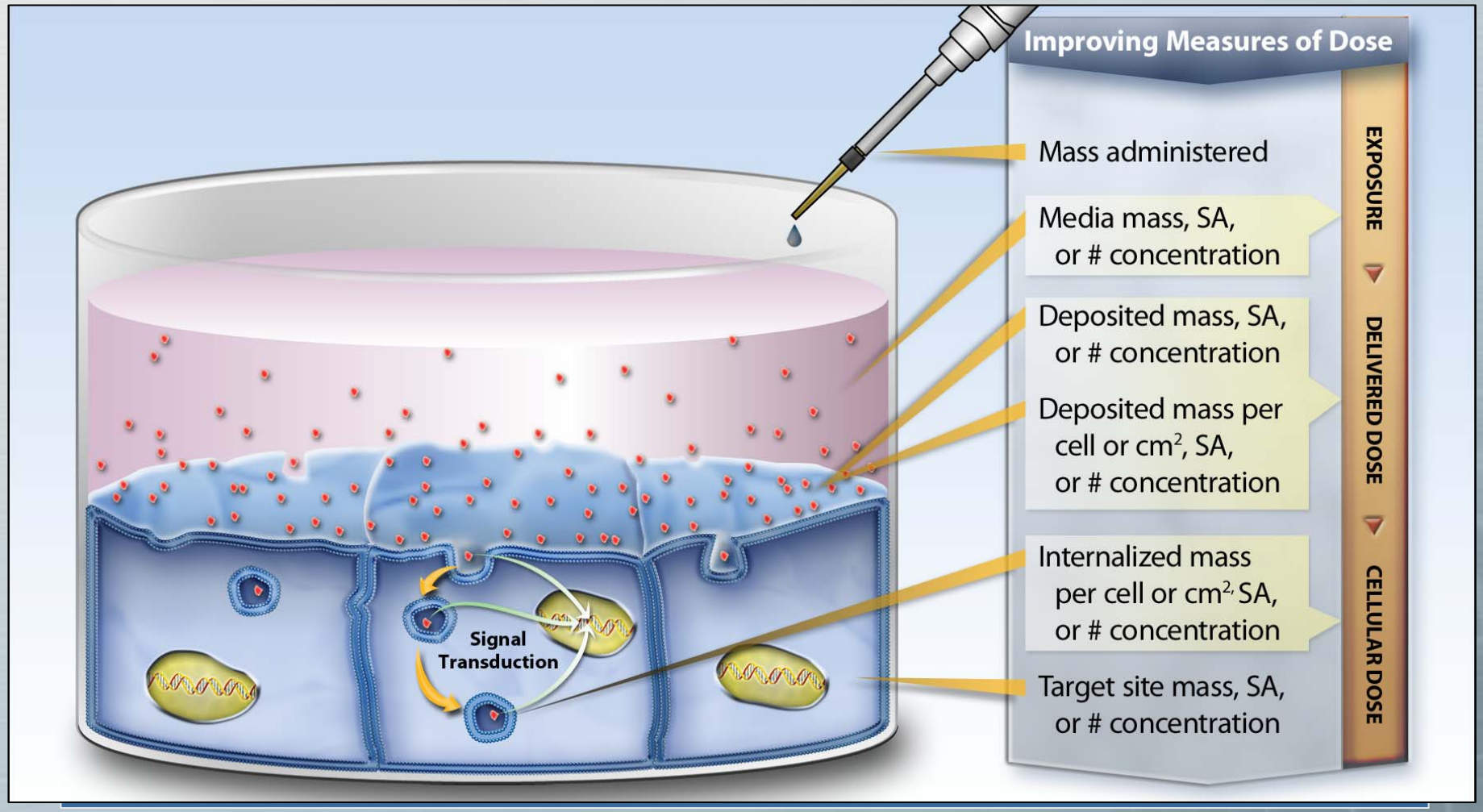
Report dose-response on a nomial media concentrations basis

Particokinetics-In Vitro

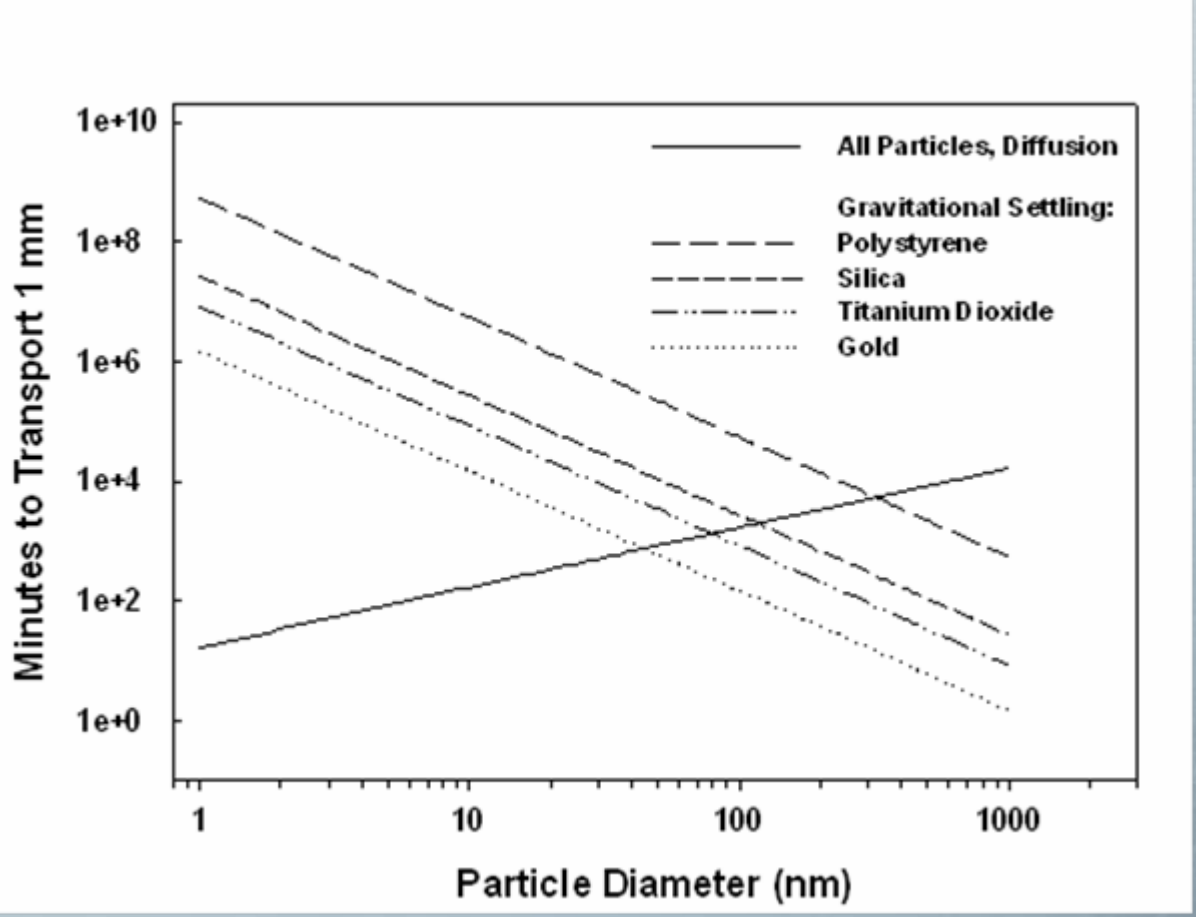
- **Shape, size, and density affect settling rate.**
- **Settling time impacts timing/magnitude of response**
- **Media "dose" is different than dose to the cell**



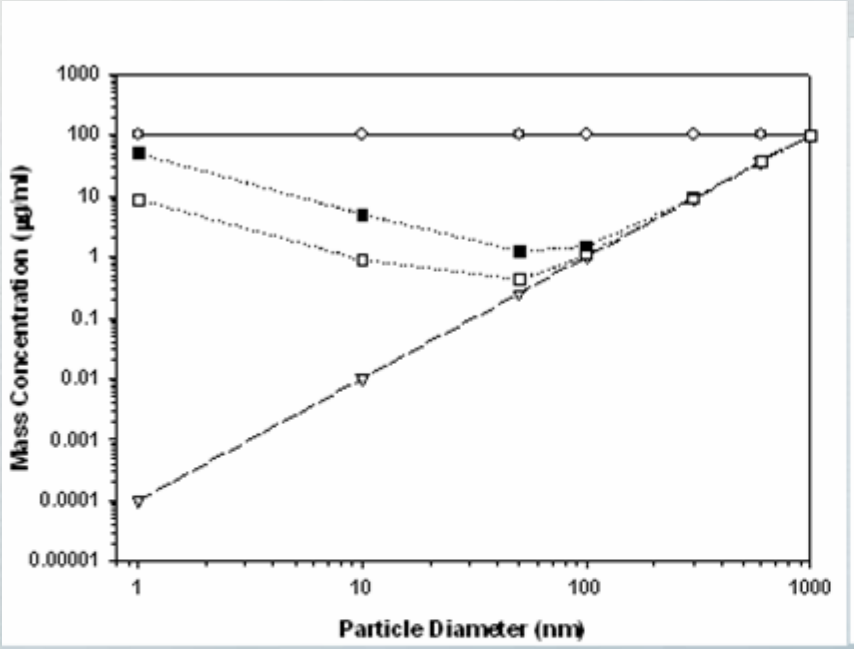
Dosimetry for Particles is Important

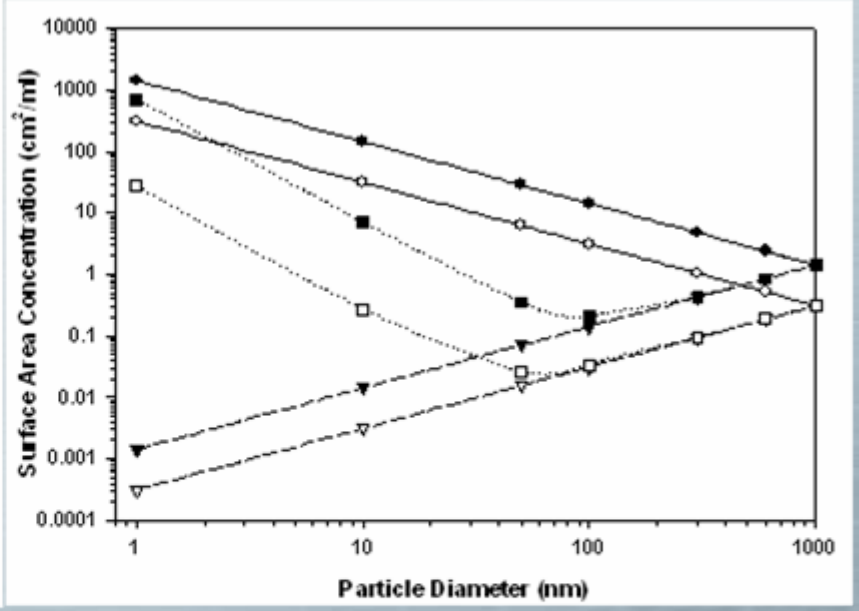


Delivery to Cells Can be Very Different



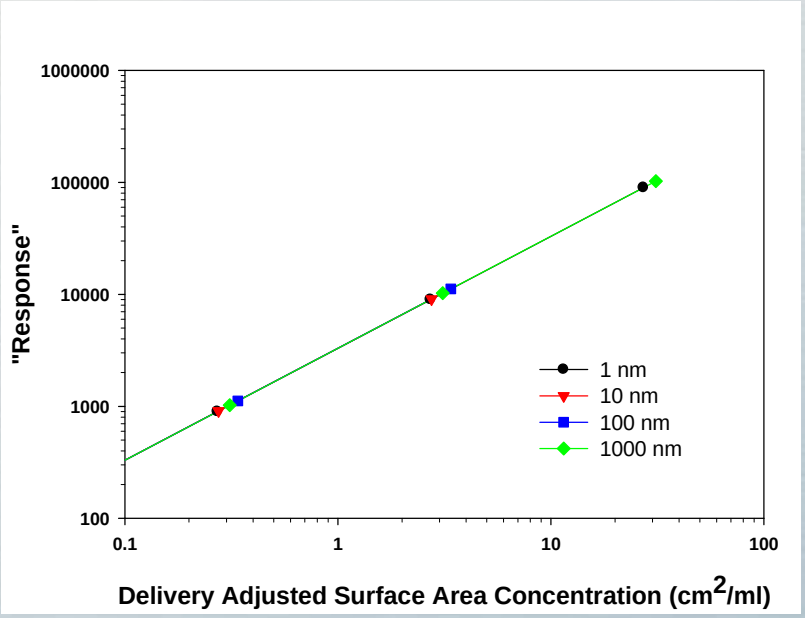
From First Principles: TiO2 and Gold Nanoparticle Dosimetry

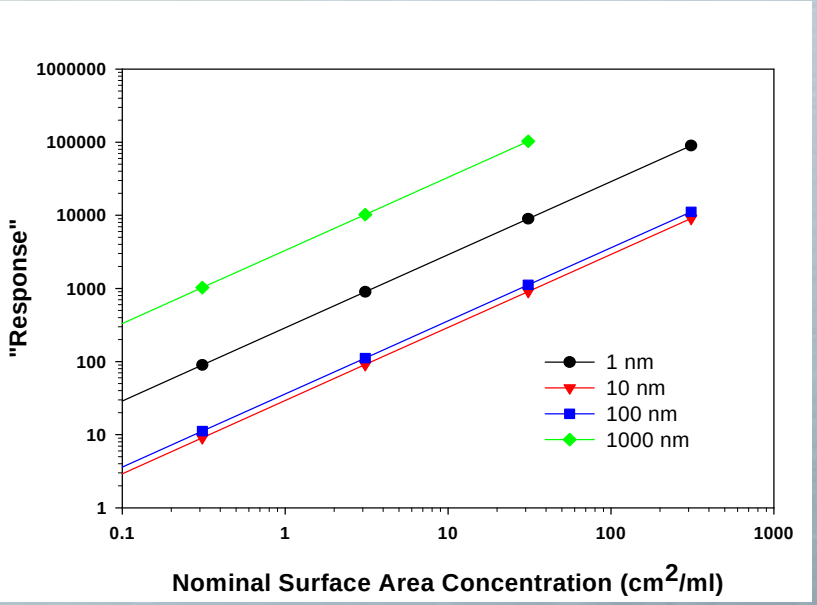




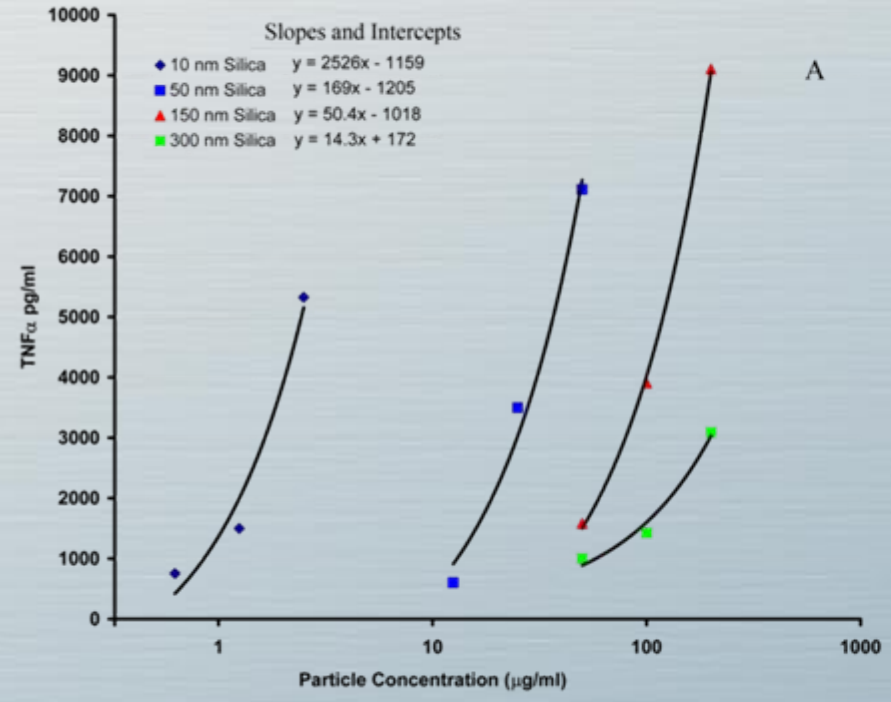
TiO2] NMC
Au
TiO2] Gravitational Settling Adjusted NMC
Au
TiO2] Gravitational and Diffusional Transport Adjusted NMC
Au

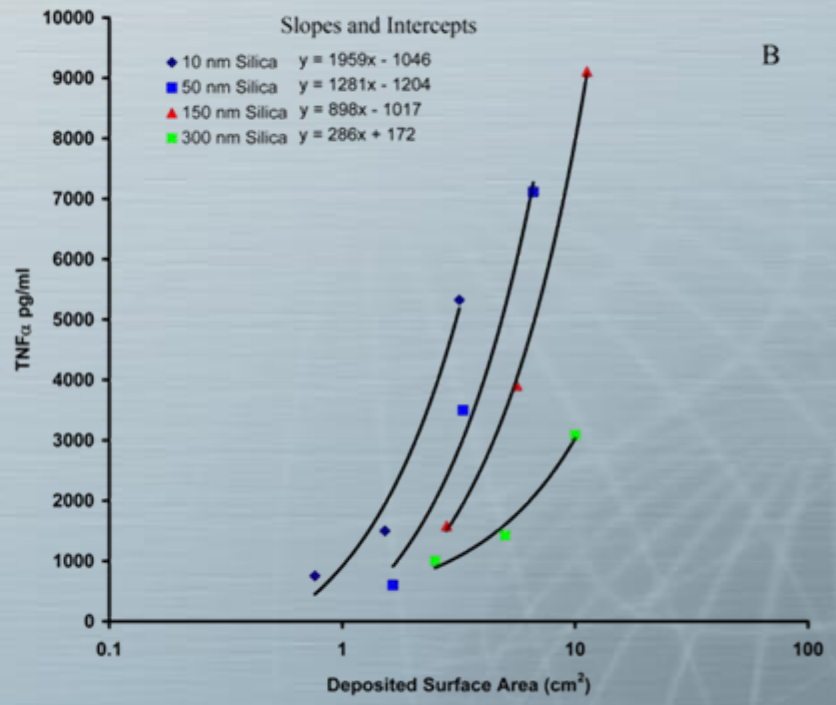
Nominal Media Concentration Obscures Underlying Dose-Response Behavior





Delivered Dose Reveals Surface Area Relationship





Carbon Nanotube Toxicity

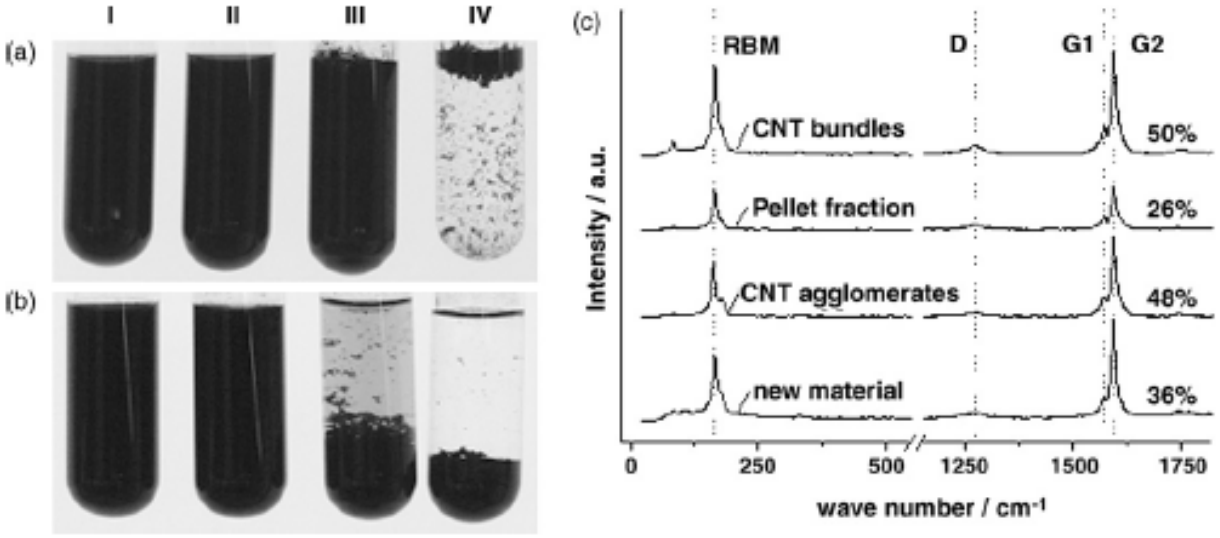
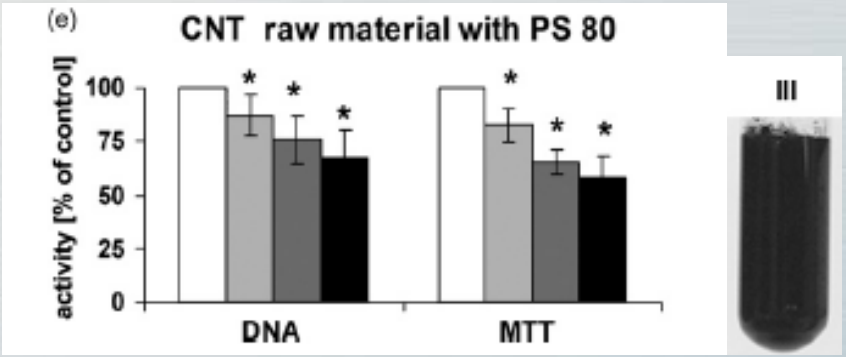
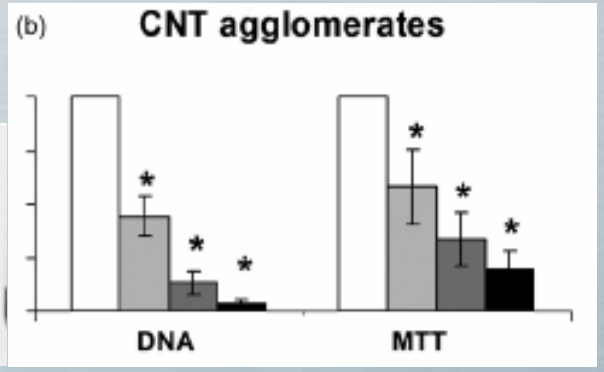
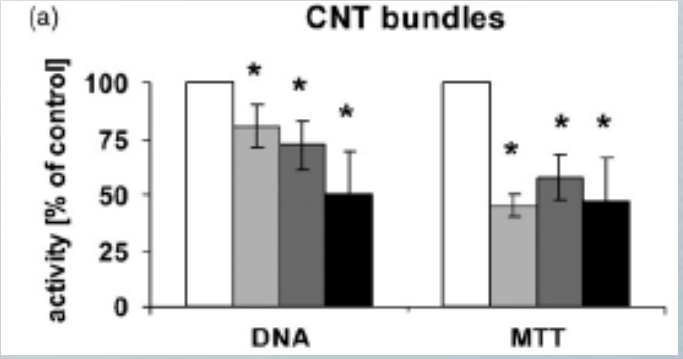


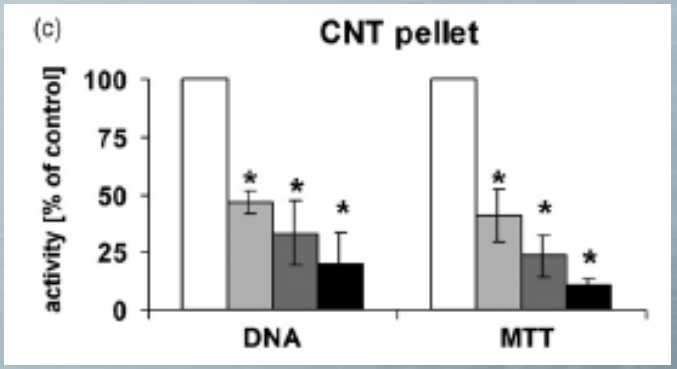
Fig. 3. CNT suspensions, Raman spectroscopy and the relative content of single wall CNTs. (a) CNT raw material and (b) CNT-agglomerates suspended in: (I) 1% SDS, (II) 100% dimethylformamid, (III) 0.002% PS80 and (IV) H_2O. (c) Corresponding Raman spectra with the relative SWCNT content [%] measured by NIR-VIS spectroscopy, values ±5% standard deviation.

Settling Impacts CNT Toxicity?









Supernatant

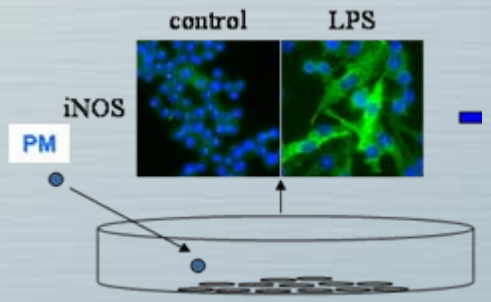
P. Wick et al. / Toxicology Letters 168 (2007) 121–131

Pellet

Slow Settling → **Rapid Settling**

Another Motivation for Understanding Dose for Nanomaterials

- Extrapolation for safety assessment!
- Dose-response data
  - in vitro to in vivo
  - animals to humans
  - normal to disease state

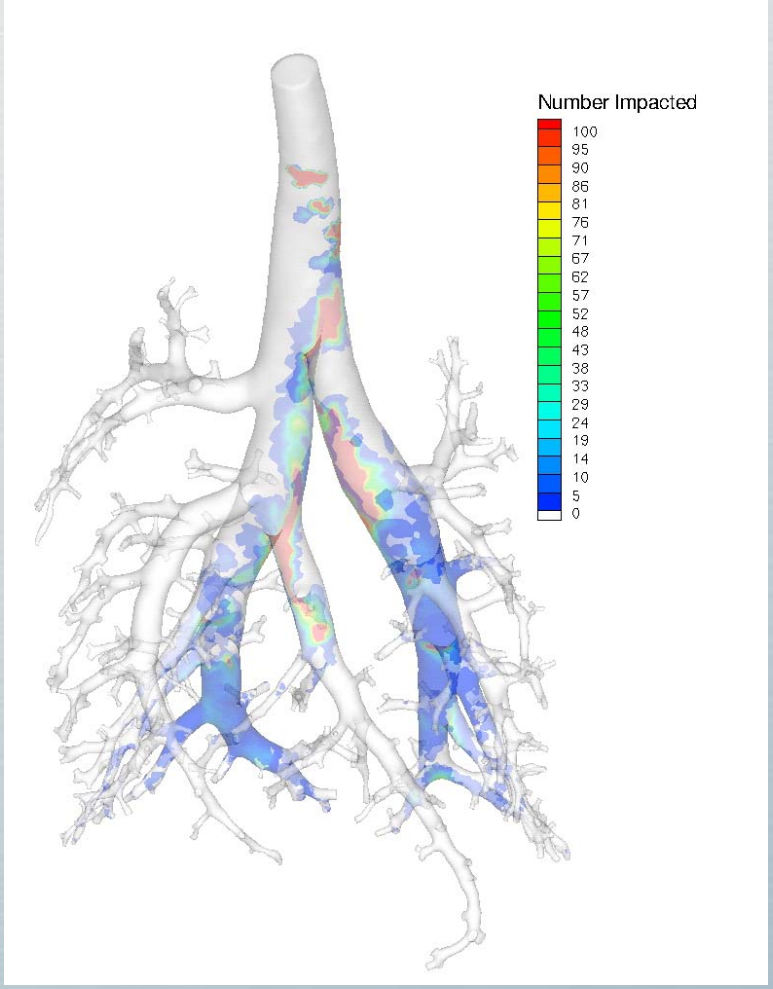


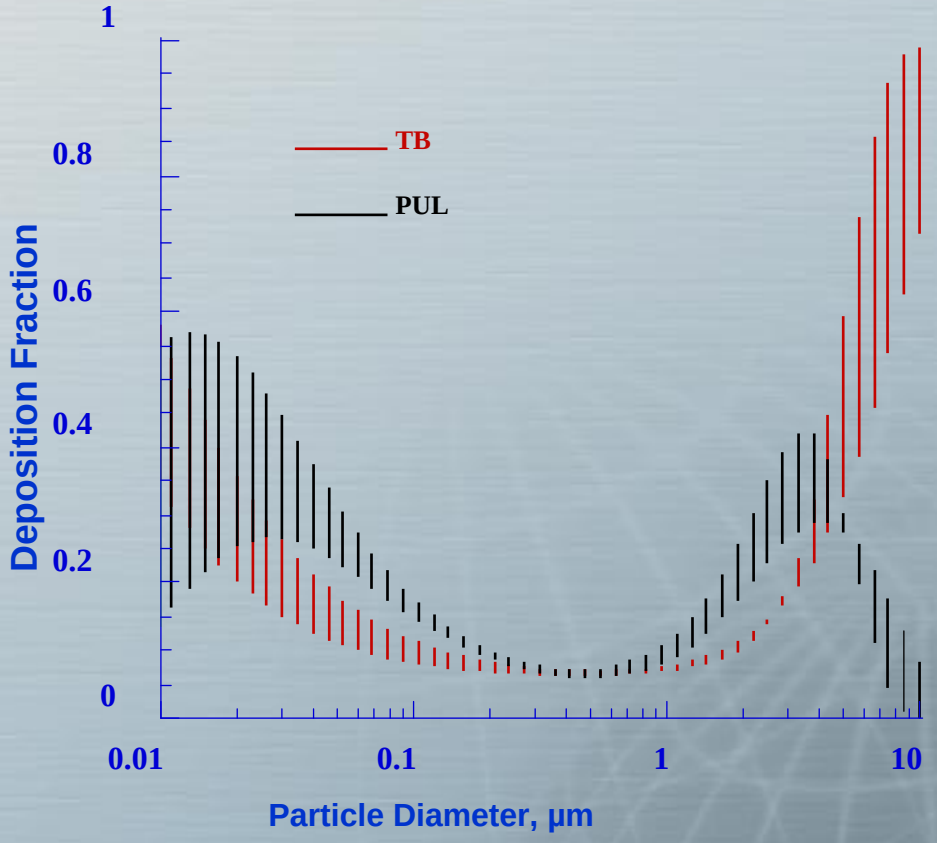
In Vitro

Human

In Vivo

Lung Dosimetry

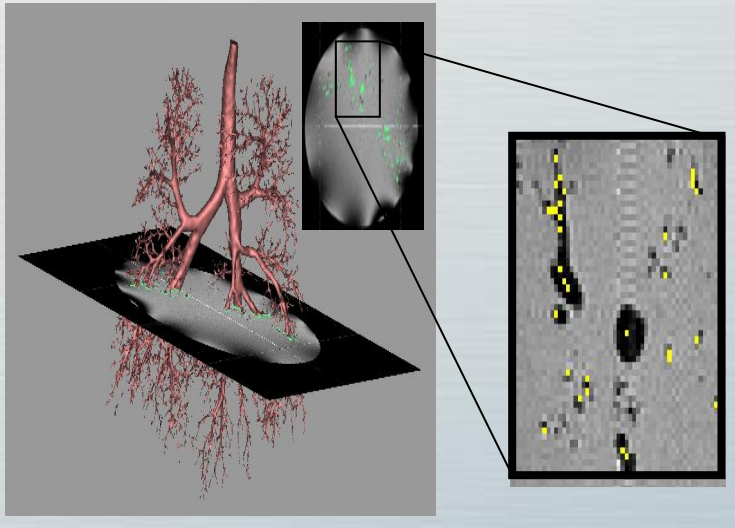




Computational Models of Particle Deposition in the Lung

- ICRP
  - Human, empirical, multicompartment, designed and validated for radionuclides
- Multi-path Particle Dosimetry Model (CIIT)
  - Rat, human, improved geometries, regional deposition, easy to use
- Virtual Respiratory Tract (PRT) Model
  - Improved 3D geometries, multiple species, structure dependent (disease state), gas phase and particles, and, eventually, cellular-level dosimetry and enzymology.

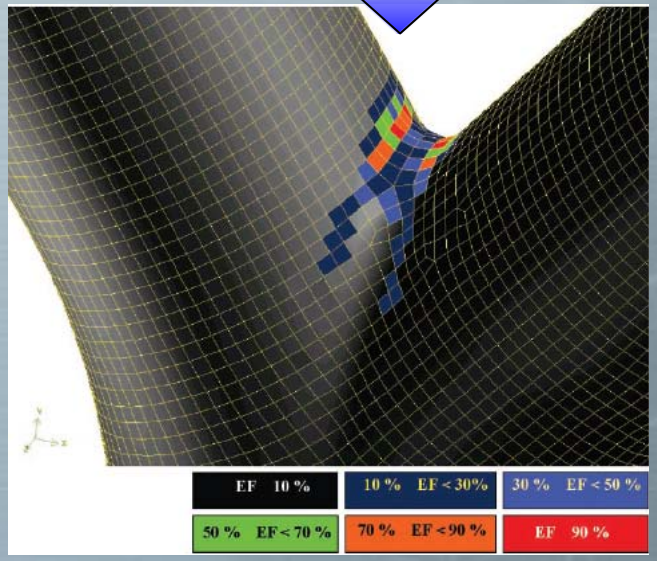
PRT Model Development



NWGrid

Computational mesh

NWPhys

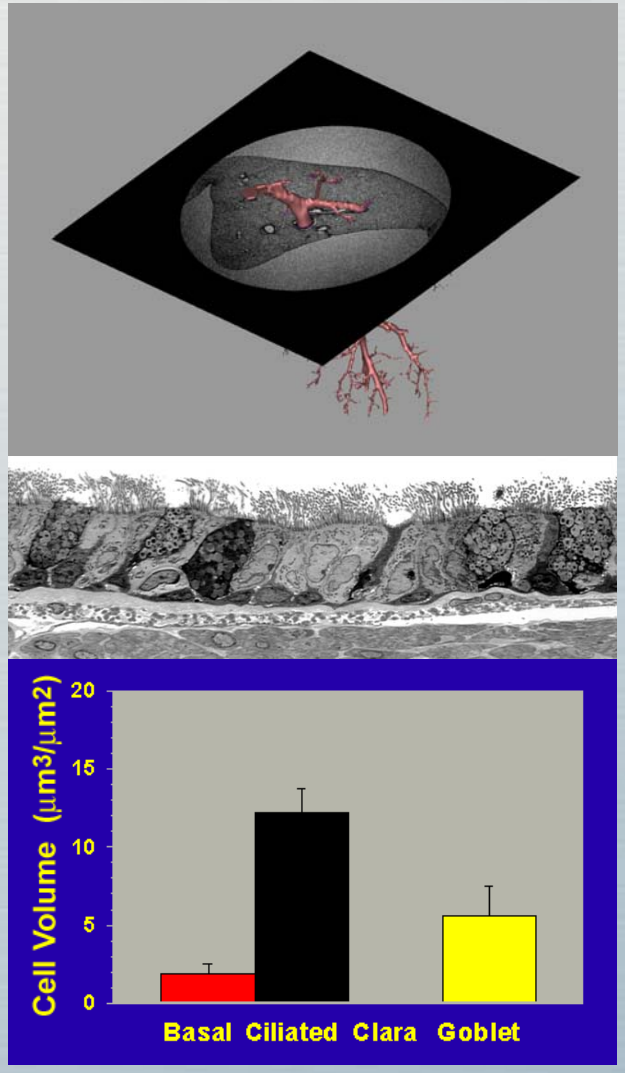


Data for Modeling:

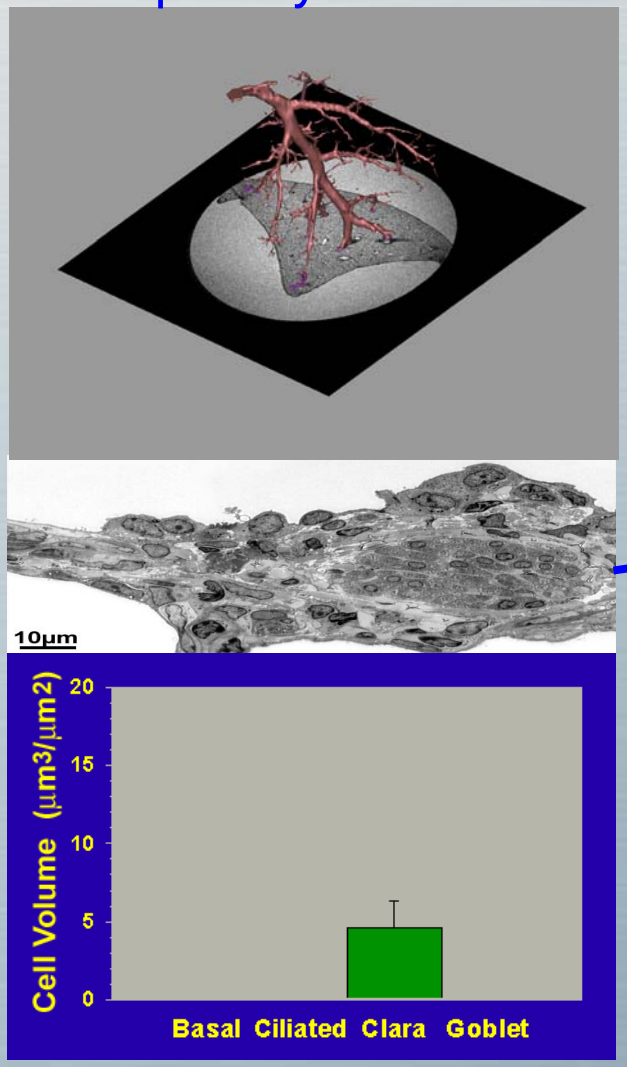
- Length
- Major and minor diameters
- Bifurcations angle
- Azimuth angle
- Airway volumes
- Airflow Splits

Fixed Lung Imaging/Histology Mapping

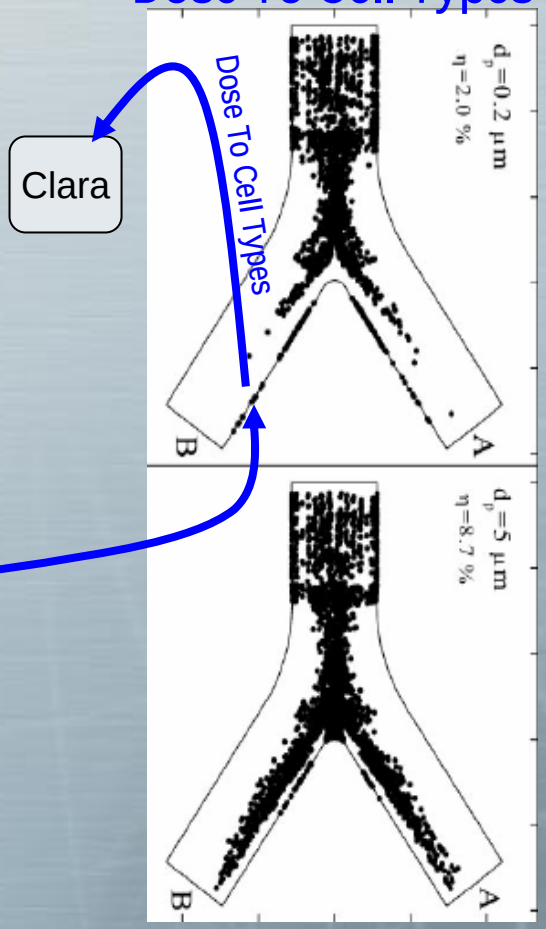
Proximal Bronchiole



Respiratory Bronchiole



Dose To Cell Types



Conclusions

- With few exceptions, there is insufficient data to support risk assessments for nanomaterials.
  - There is a great deal of work to be done
- Our greatest concern is that funding limits will hamper risk assessment
  - Fundamental research: NIH/NIEHS/EPA/NSF
  - Applied Research/Hazard Assessment: NTP
  - Bridging research..???????
- Research should focus on:
  - Integrated assessments of materials with significant potential for environmental release/human exposure
  - Dosimetry
  - Fundamental material characteristics driving toxicity

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