Current knowledge of nanomaterial toxicity

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Outline

- Introduction to the NTP
- Nanotechnology and nanomaterials
- Are all nanomaterials the same?
- How do you assess safety/toxicity?
- Why would nanomaterials be different?
- Routes of exposure and kinetics
- Dose metrics for assessing dose response
- Modes of action
- Strategies and pitfalls
- Safe handling of nanomaterials
- Take home key issues
National Toxicology Program (NTP)

- Not a “regulatory” agency
- Established in 1978
  - To coordinate toxicology research in DHHS
  - Headquartered at NIEHS
- Research on submitted “nominations”
  - Submitted by public, govt agencies, academia
- GLP compliant “testing”
  - CRO contracts, not research grants
  - Thousands of environmental and industrial chemicals, pharmaceuticals, etc. evaluated in comprehensive toxicology studies
- Risk assessment activities
  - Report on Carcinogens
  - CERHR
  - Validation of alternate models

dept.of.healthousandhuman.services.gov

Dept of Health and Human Services (DHHS)

NIH

CDC

FDA

NIEHS

NIOSH

NCTR

DERT

DIR

NTP
NTP research areas

- 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
Nanotechnology:

The intentional manipulation of matter at the nanoscale (1-100nm), to create materials and products with nanostructure-dependent properties.

The term nanotechnology encompasses the technologies used to manipulate and characterize nanostructures, as well as the resulting materials and products.
Desirable Applications of Nanotechnology

1. “Smart” therapeutics
2. Targeted molecular imaging agents
3. Biological sensors/diagnostic tools
4. Tissue engineering
5. Nano-enabled products
Early fears

• Self replicating nanobots
  – “Grey goo” scenario

• Past examples of “technology gone wrong”
  – Genetically Modified Organisms (GMO)
  – Ethyl lead
  – Asbestos

• “Fear of the unknown”
“Early” studies on showing toxicity of nanotubes

- Carbon nanotubes
- Lung granulomas after intratracheal instillation in rats and mice
  - Warheit et al 2003
  - Lam et al 2003
  - Reaction to foreign particulate
- Supported by later studies
  - Mueller et al 2005
    - MWCNT
  - Shvedova et al 2006
Are all nanomaterials the same?
Diversity of nanoscale materials

- Single and multi walled nanotubes
- Fullerenes
- Nanoshells
- Metal oxides
- Dendrimers
- Quantum dots
- Nanosomes
Real world nanomaterial examples

- Multiwalled Carbon Nanotubes
- Fullerene C60 aggregates
- Anatase TiO2
- Rutile TiO2
Can you “classify” nanomaterials

<table>
<thead>
<tr>
<th>Primary Size</th>
<th>Shape</th>
<th>Surface</th>
<th>Composition</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoscale (1-100nm)</td>
<td>Spheroid</td>
<td>Neutral</td>
<td>Homogeneous</td>
<td>Monodispersed</td>
</tr>
<tr>
<td>Low nano (1-10)</td>
<td>Fibrous</td>
<td>Anionic</td>
<td>Heterogeneous</td>
<td>Aggregated</td>
</tr>
<tr>
<td>Mid nano (10-30)</td>
<td>Tubular</td>
<td>Cationic</td>
<td>Structured</td>
<td>Nanostructured</td>
</tr>
<tr>
<td>High nano (30-100)</td>
<td>Amorphous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-micron (100-1000nm)</td>
<td>Amorphous</td>
<td>Hydrophobic</td>
<td>Amphiphilic</td>
<td></td>
</tr>
</tbody>
</table>
How do you assess safety?
Safety = lack of risk
Risk = hazard x exposure

- Exposure assessment
- Hazard identification
- Hazard characterisation
- Dose-response
Areas of emphasis to assess risk

• Exposure and dose metrics
  – How do we measure exposure?

• Internal dose-Pharmacokinetics in biological systems
  – What physiochemical properties determine the absorption, distribution and elimination of nanomaterials?

• Early biological effects and altered structure function
  – What physiochemical properties determine biocompatibility?

• Adverse effects
  – What are the critical determinants of toxicity for those that are toxic?
Why would nanomaterials be different?
General concerns over nanoscale vs microscale materials

• Routes of exposure may differ
  – Different portal of entry and target cell populations

• Different kinetics and distribution to tissues
  – Due to size or surface coating/chemistry

• Higher exposure per unit mass
  – Biological effects may correlate more closely a surface area dose metric

• Unique properties = unique modes of action?
Routes of exposure may differ
Increased uptake of nanoscale vs microscale particles

- Uptake of polystyrene microspheres
  - 50, 100, 300, 500, 1000 and 3000 nm
  - Oral administration to female SD rats
- Increased uptake as size decreases
- Materials may become bioavailable as size decreases
  - Increased opportunity for effects

Jani et al 1990.
Size determines sites of deposition within the lung

Figure 8. Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar region of the human respiratory tract during nose breathing. Based on data from the International Commission on Radiological Protection (1994). Drawing courtesy of J. Harkema.
Metal oxides

• Examples
  – Titanium, Zinc, Iron, Cerium

• Desirable properties
  – Transparent
  – UV absorbing properties
  – Catalytic properties

• Uses
  – Cosmetics/Sunscreens
  – Catalysts, remediation, self cleaning windows
  – Chemo-mechanical polishing agents for semiconductor wafers

• Research issues
  – ROS production and macromolecule damage
  – Crystal type/size
  – Coatings
Penetration of skin by Quantum dots?

- Observed in vitro-pig skin
  - Ryman-Rasmussen et al 2006
  - 14-35 nm hydrodynamic diameter
  - Flow through diffusion cell system

- Not seen in mouse in vivo
  - Gopee et al 2007
  - Dermal exposure to SKH-1 mice
    - (CdSe)CdS)-PEG (Emax621nm)
    - No penetration through intact skin
Lack of penetration of titanium dioxide through human skin

- Mavon et al 2007
- Human volunteers
  - 20nm trimethyloctylsilane coated TiO2 in an oil water emulsion
- Recovery of most of the TiO2
  - Supported by TEM analyses

<table>
<thead>
<tr>
<th></th>
<th>MBBT</th>
<th>TiO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in vitro</td>
<td>in vivo</td>
</tr>
<tr>
<td></td>
<td>in vitro</td>
<td>in vivo</td>
</tr>
<tr>
<td>Applied dose</td>
<td>178 ± 4.7</td>
<td>167 ± 7.4</td>
</tr>
<tr>
<td>SC, 15 tape stripings</td>
<td>142 ± 6.4</td>
<td>154 ± 14</td>
</tr>
<tr>
<td>Epidermis</td>
<td>16.2 ± 6.1</td>
<td>/</td>
</tr>
<tr>
<td>Dermis</td>
<td>0.5 ± 0.2</td>
<td>/</td>
</tr>
<tr>
<td>Receptor fluid</td>
<td>LOD</td>
<td>/</td>
</tr>
<tr>
<td>Total recovery</td>
<td>159 ± 6</td>
<td>154 ± 14</td>
</tr>
<tr>
<td>% of applied dose</td>
<td>89.3</td>
<td>92.2</td>
</tr>
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Means ± SD, n = 3, data expressed in μg/cm². n.d. = Not detected.
Different kinetics
Differences in kinetics and tissue distribution for Qdots

- Fischer et al 2006
- Male SD rats; i.p injection.
- BSA vs lysine/mercaptoundecanoic acid coated
- Longer half life for uncoated Qdot
Higher exposure per unit mass
Dose metrics are generally mass based

- Ambient concentrations in air, water, food
- Intake/exposures
  - Eg mg/kg, ppm in air,
- Dose rate
  - Eg mg/kg/day, lifetime adjusted daily dose
- Tissue concentration
  - Eg mg/kg tissue weight, pg/g blood lipid, metabolite concentration
- Cumulative exposure
  - Eg Area under the curve (AUC)
- Window of exposure
  - Eg mg/kg on gestational day 5
- Biological effective dose
  - Eg Concentration of DNA adducts
Surface area metrics

- 1μm cube (e.g. respirable particle)
  - Surface area of = 6μm²

- 100nm cube
  - 1000 cubes is equivalent volume (mass)
  - Surface area of equivalent mass = 60 μm²
  - 10x more surface area

- 10nm cube
  - 1,000,000 cubes
  - Surface area of equivalent mass = 600 μm²
  - 100X more surface area

- e.g. a nanomaterial could require a 10-100x lower inhalation exposure limit.
Surface area metrics: A key consideration

- Particle number-based and surface area-based metrics increase with decreasing particle size
- Mass-based potency may differ, but surface area-based potency may not
- Requires studying particles of similar composition but varying particle size, coatings, shape or other physicochemical parameter
NIOSH Current Intelligence Bulletin for Titanium Dioxide

- Draft Recommendations for Occupational exposures

- Current limits
  - 1.5 mg/m³ (respirable dust)
  - 15 mg/m³ (total dust)

- NIOSH recommendations
  - 1.5 mg/m³ for fine TiO₂
  - 0.1 mg/m³ for ultrafine (nano)
    - 15X lower level
  - TWA for up to 10 hr/day during a 40-hour work week.

- Due in part to higher particle surface area for ultrafine
Unique properties = unique mode of action?
Protein fibrillation in vitro induced by nanoparticles

• Linse et al 2007, PNAS 104,8691

• Induction of b2-microglobulin protein fibril formation in vitro
  – Surface assisted nucleation

• Observed with multiple NPs
  – 70, 200 nm NIPAM/BAM NPs
  – 16nm Cerium oxide NPs
  – 16nm quantum dots
  – 6nm dia MWCNTs

• Fibril formation is implicated in development of human disease
  – Alzheimer's
  – Creutzfeldt-Jakob disease
  – Dialysis related amyloidosis
Strategies and pitfalls
Biological levels and hazard evaluation strategies

- Immediate Human Relevance
- Mechanisms
Consideration of “particokinetics” for in vitro studies

- Teeguarden et al 2007
- Diffusion, settling and agglomeration in vitro may impact interpretation of findings in vitro.
- Dose metrics may be different when particokinetics are considered.
- Mathematical modeling approaches may be able to compensate.
Carbon Nanotubes- case study

- In composites for structural integrity
- Field emitter sources in flat panel displays
- X-ray machines
- Fuel cells
- Nano-electronics
- Nano-medicine/drug delivery
- Bullet proof textiles
- Cables for NASA space elevator
Nanotube-toxicity

• Lung granulomas after intratracheal instillation
  – Reaction to foreign particulate
  – Associated fibrosis

• Single walled
  – Warheit et al 2003
  – Lam et al 2003
  – Shvedova et al 2006

• Multiwalled
  – Muller et al 2005
“Asbestos like” activity of long MWCNT

- Poland et al 2008
  - Nature Nanotech 3:423

- Injection to C57Bl/6 mice
  - 50ug or vehicle into peritoneal cavity
  - Evaluation at 7 days

- Pathology
  - Inflammation
  - Foreign body Giant Cells
  - Granulomas

- Long MWCNTs and long fibre amosite (LFA) gave similar responses

- Tangled MWCNT gave different responses
Lack of short term pulmonary toxicity of MWCNT

- Mitchell et al 2007
  - Inhalation study in C57BL/6
  - Observed up to 14 days
  - 6hr/d, 7 d/wk, whole body
  - up to 5mg/m³: 10-20nm x 5-15um

- Results
  - No inflammatory response or pathology
  - Induction of IL10 and NQO1
  - Suppressed PFC in SRBC assay
Safe handling of nanomaterials
Working with Engineered Nanomaterials

- Nanomaterial-enabled products such as nanocomposites and surface coatings and materials comprised of nanostructures are unlikely to pose a risk of exposure during their handling and use.

- Processes used in their production may lead to exposure to nanoparticles.

- Processes generating nanomaterials in the gas phase, or using or producing nanomaterials as powders or slurries/suspensions/solutions pose the greatest risk for releasing nanoparticles.

Baron et al 2003

Figure 5. Summary of single component vortex-shaker fluidized bed generation of HPCO® SWCNT. Data have been smoothed for clarity.
Contexts for use and exposure to nanoscale materials

- **Class I- Bulk**
  - A-One phase in bulk
  - B-Multiphase

- **Class II- Surface**
  - A-Structured surface
  - B-Film
  - C-Structured films

- **Class III-Particles**
  - A-Surface bound
  - B-Suspended in liquids
  - C-Suspended in solids
  - D-Airborne

Hansen et al 2007
Safety in research

- Nanomaterials generally not handled differently than any other chemical/particle of unknown hazard
- Standard laboratory safety rules apply
- Personal Protective Equipment
  - Lab coats, gloves, safety glasses
- Engineering controls
  - Glove boxes
  - Hoods for handling of all open materials
  - Balances in the hoods for weighing materials
- Waste
  - Handled as hazardous-Incineration
  - Wet wiping for spills
- Occupational health surveillance unit
  - Monitors health of workers
Fullerene C60: rodent inhalation studies

- Assumption that it is hazardous
- Hoods for parts of the system that must be handled
- Glove box for C60 material in reservoir for generation system
- Full-face respirator in animals rooms during loading, unloading, cleaning operations.
  - Survivair 1058 multicontaminant cartridges
    - P100 (HEPA)
NIOSH-Approaches to Safe Nanotechnology:

- Current draft for public comment
  - July 2006
- Website:
  - www.cdc.gov/niosh/topics/nanotech
- Raise potential safety and health concerns from exposure to nanomaterials and addresses current and future research needs.
Respirators

- May be necessary when engineering and administrative controls do not adequately prevent exposures.
- Decision should be based on professional judgment and
  - toxicity information
  - exposure measurement data,
  - frequency and likelihood of the worker's exposure.
- Preliminary evidence
  - no deviation from the classical single-fiber theory for particulates as small as 2.5 nm in diameter.
- NIOSH certified respirators likely will be useful for protecting workers
  - when properly selected and fit tested as part of a complete respiratory protection program.

NIOSH-Approaches to Safe Nanotechnology- July 2006
Real time monitoring

- Limited sensitivity to detect small particles.
- Relatively few techniques exist to monitor exposures with respect to aerosol surface area.
- Nanoparticles are ubiquitous in many workplaces
- Difficult to differentiate between incidental and process-related nanoparticles using number concentration alone.
Precautionary measures

• Implement risk management program in workplaces to minimize potential exposures

• Use engineering control techniques
  – source enclosure (i.e., isolating the generation source from the worker) and local exhaust ventilation

• Use good work practices
  – cleaning of work areas using HEPA vacuum pickup
  – wet wiping methods
  – protective clothing/respirators

• Consider need for occupational health surveillance program
Grand Challenges for responsible nanotechnologies

Maynard et al 2006, Nature 444, 267
Key issues for the field of “nanotoxicology”

• “Are nanomaterials safe?” = “Are chemicals safe?”
  – There is no single type of nanomaterial

• Effects can scale with surface area
  – Paradigm shift in how we estimate “dose” for assessing risks relative to other agents.

• Lack of adequate characterization of what a given “test article” is
  – Major obstacle to developing structure-activity relationships

• Nanoscale phenomena occurs at the interface between chemical space and physical space.

• Very limited information on exposures
“An Englishman’s never so natural as when he’s holding his tongue.”

Henry James