NTP
National Toxicology Program

Evaluating Human Health Risks from Nanomaterials

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National Institute of Environmental Health Sciences/National Institutes of Health

National Toxicology Program (NTP)

- A cooperative, interagency, federal resource for toxicological sciences
- Established to evaluate substances of public health concern through the development and application of the tools of modern toxicology and molecular biology
- Charged to develop approaches and generate data to strengthen the scientific basis for risk assessments
- Structured to address issues and problems on a scale not easily duplicated by others
- Participant- National Nanotechnology Initiative (NNI), Nanomaterials Environmental and Health Implications (NEHI) Working Group

Current NTP Research Areas

- Cellular phone radiation
- Dietary supplements
- DNA-based therapeutics
- Drinking water contaminants
- Endocrine disruptors
- Green chemistry
- Herbal medicines
- Nanoscale materials
- Occupational exposures
- Persistent organic pollutants
- Phototoxicology

Technical and Scientific Challenges of Nanotechnologies

- What constitutes "nano"?
  - Manufactured versus natural
  - Exhibit quantum properties?
  - Are agglomerates of nanoscale materials still nano?
- Why might nanomaterials be toxic?
- What are the critical determinants of toxicity for those that are toxic?
- How do we measure relevant exposures? Hazard x Exposure= Risk
- Given the vast number of potential nano substances, can we keep up?

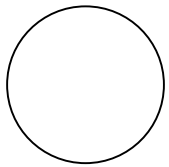
<u>Key Issue</u>

Does what we've learned so far in toxicology apply to nanomaterials?

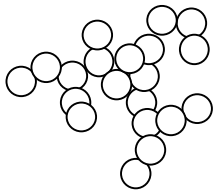
What makes "nano" different?

Particle Categories

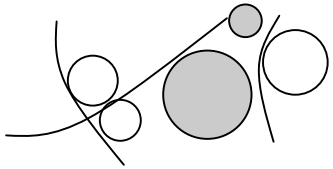
Classes of engineered nanoparticles



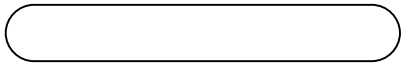
A. Spherical homogeneous



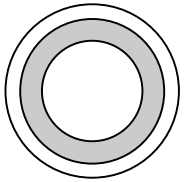
D. Agglomerate homogeneous



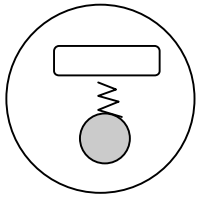
G. Heterogeneous agglomerate



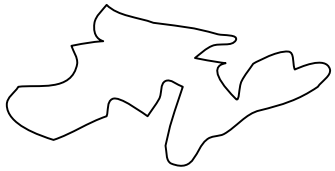
B. Fibrous homogeneous



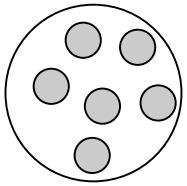
E. Heterogeneous concentric



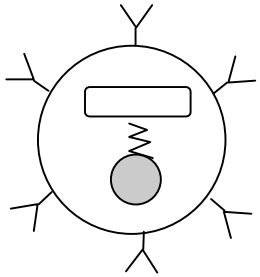
H. Active particle



C. Non-spherical homogeneous



F. Heterogeneous distributed



I. Multifunctional particle

Where Do We Start?

- Approach

	Chemical/ particle	Nanomaterial
– *Can we reasonably predict?*		
– Structure/ properties	yes	no
– Absorption	yes	no
– Distribution	often	no
– Metabolism	often	sometimes
– Excretion	often	no
– Toxicity	often	no
– Target organs	often	no
– Persistence/reversibility of effect	often	no
– *Do we know how to design, perform and interpret studies?*		
– Material preparation	yes	no
– Study designs	yes	no
– Interpretation	yes	yes

Pulmonary Deposition as a Function of Particle Size

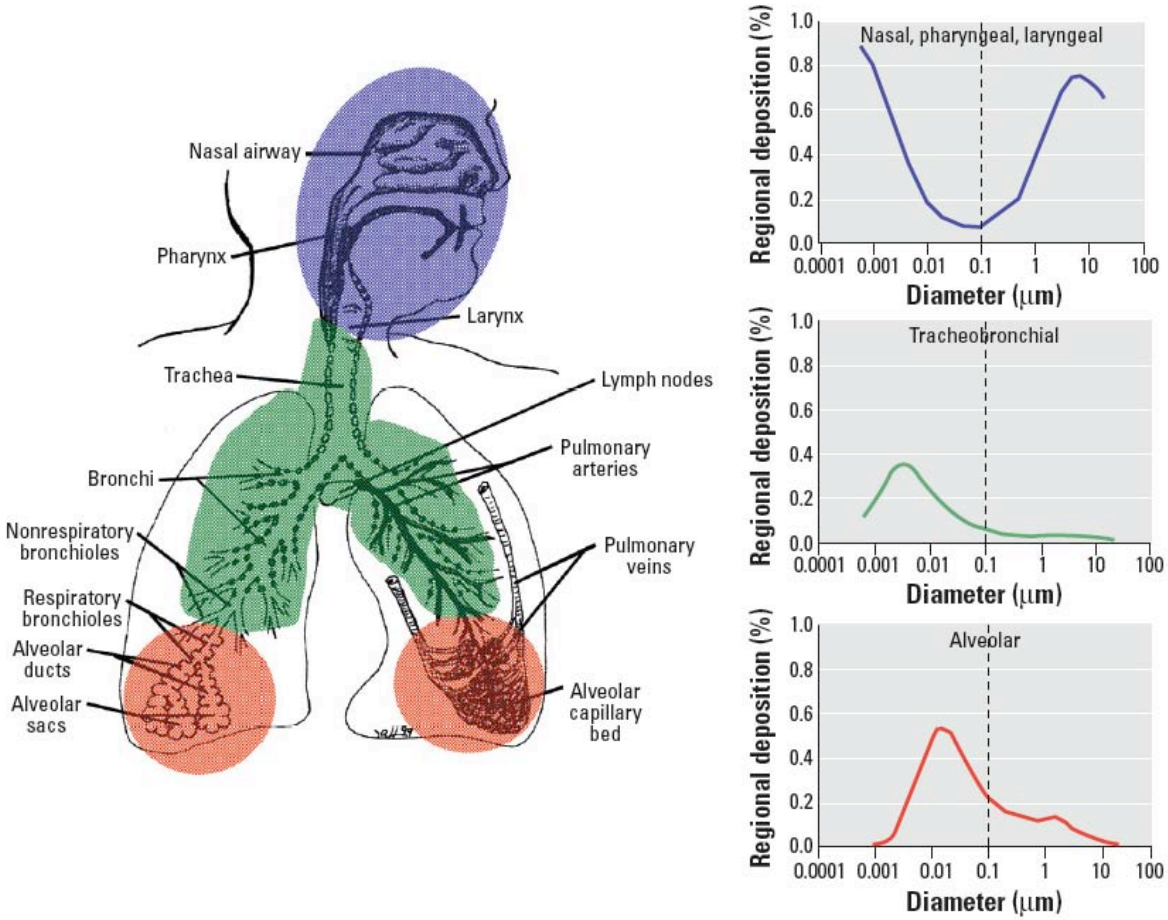


Figure 8. Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar region of the human respiratory tract during nose breathing. Based on data from the International Commission on Radiological Protection (1994). Drawing courtesy of J. Harkema.

Is Surface Area a Critical Metric?

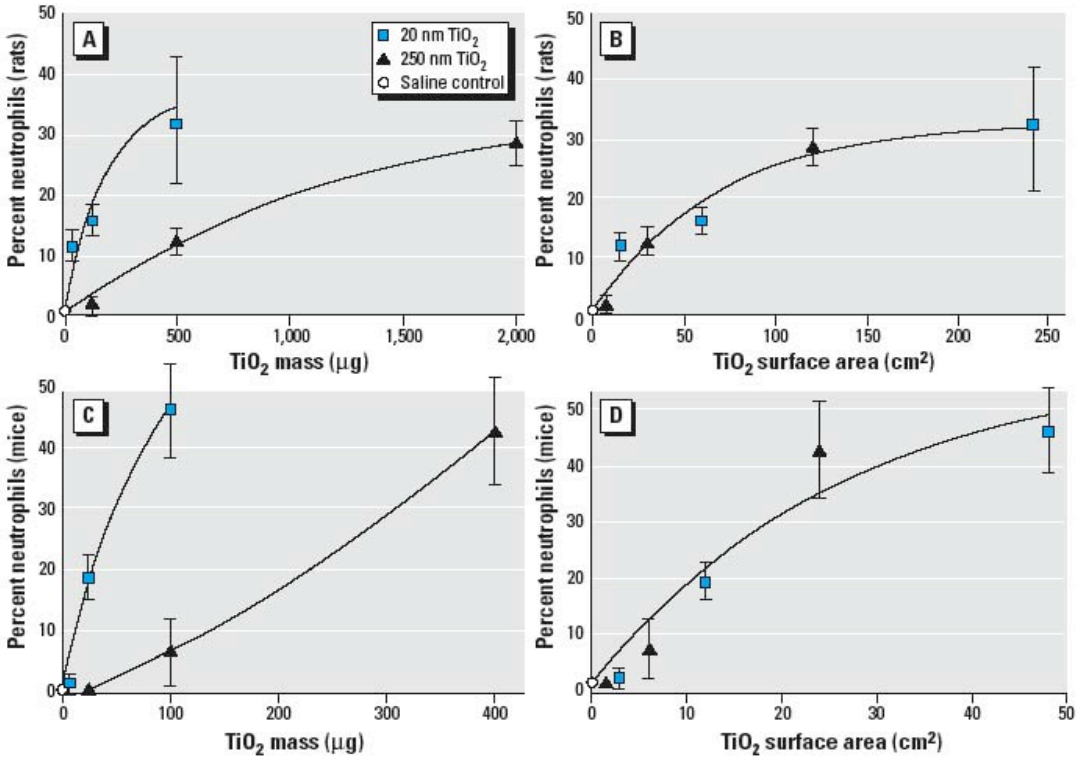


Figure 4. Percentage of neutrophils in lung lavage of rats (*A,B*) and mice (*C,D*) as indicators of inflammation 24 hr after intratracheal instillation of different mass doses of 20-nm and 250-nm TiO_2 particles in rats and mice. (*A,C*) The steeper dose response of nanosized TiO_2 is obvious when the dose is expressed as mass. (*B,D*) The same dose response relationship as in (*A,C*) but with dose expressed as particle surface area; this indicates that particle surface area seems to be a more appropriate dosemetric for comparing effects of different-sized particles, provided they are of the same chemical structure (anatase TiO_2 in this case). Data show mean ± SD.

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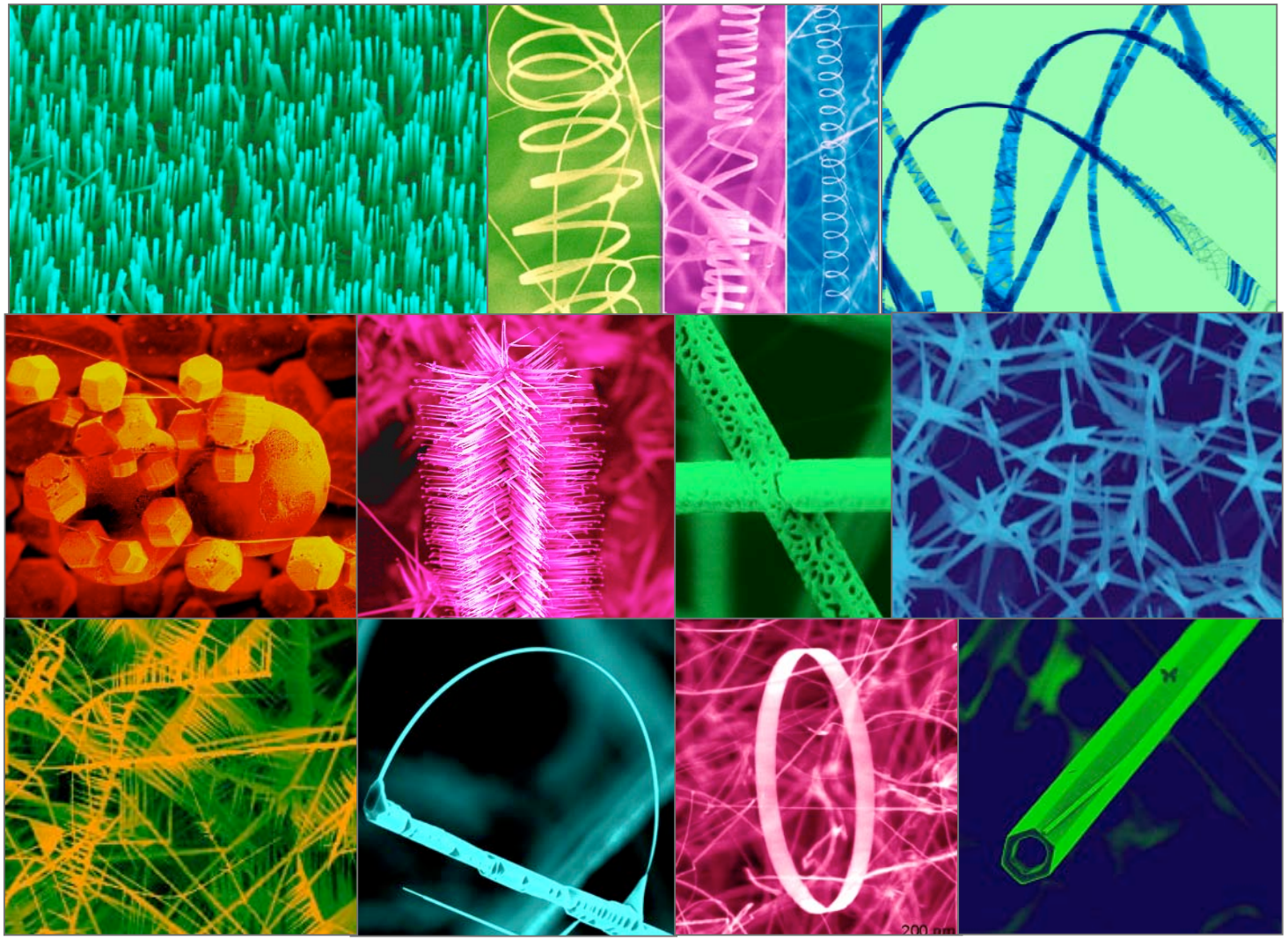


Wang et al., Materials Today, 2002

Why Might Nanomaterials be Toxic?

1) Wide range of materials can be made “nano”
2) Nanometer range is where life processes happen
3) Inhaled ultrafine particles toxic to lung and cardiovascular system
4) Transported easily, go unexpected places
5) Accumulate in cellular organelles
6) Surface coatings are critical
7) Some components are toxic as chemicals

NTP Nanotechnology Safety Initiative

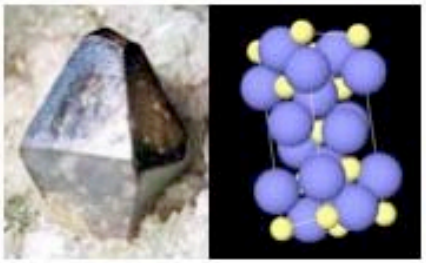
Strategic Approach:

1) Examine how nanomaterials enter, travel through, and deposit in the body
   a) Develop and apply appropriate methods to measure nanomaterials in tissues
   b) Discover key attributes that govern intake, deposition, and elimination

2) Identify key components that govern nanomaterial safety
   a) Evaluate a cross-section of size, surface coatings, and physico-chemical properties
   b) Use these as model nanomaterials for extensive toxicological evaluations

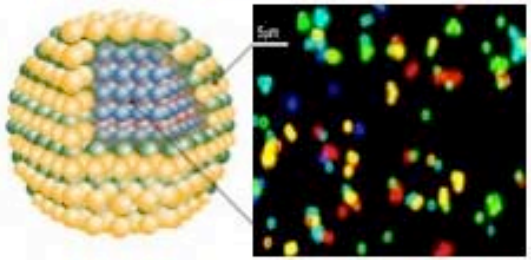
"Classes" under evaluation

Metal oxides

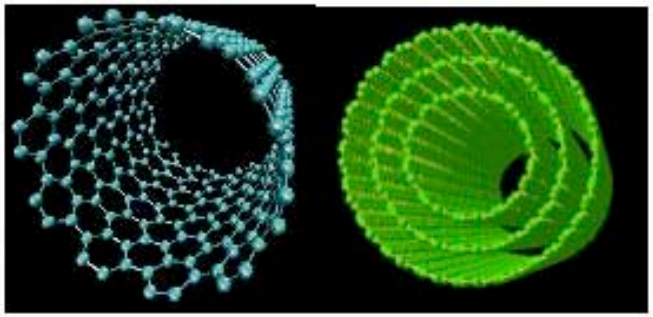


Fullerenes

Core-shells



Nanotubes

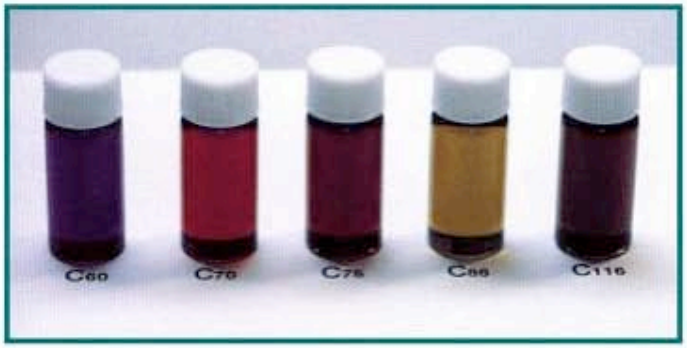


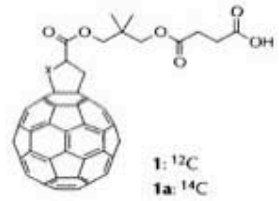
Initial Areas and Goals of NTP Studies

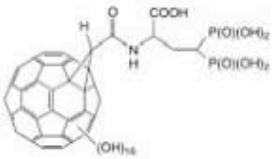
- Examine pulmonary and dermal toxicity of fullerenes
- Use quantum dots to investigate physicochemical parameters (size, shape, surface coatings) that affect oral absorption, intravenous distribution and dermal penetration and pharmacokinetics
- Evaluate physicochemical characteristics of metals oxides in sunscreens
- Evaluate dermal penetration of metal oxides in sunscreens
- Evaluate potential photocarcinogenicity of titanium dioxide

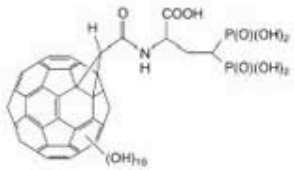
Spectrum of Fullerenes

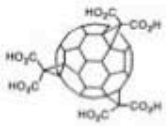
- Pure fullerenes
  - C_{60}, C_{70}, C_{76}, C_{86}, C_{116}.....
- Metal endohedrals M@C60
  - Metals encased by fullerene
- Surface modified fullerenes
  - Malonic acid derivatives
  - Hydroxylated fullerenes

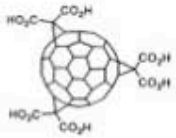


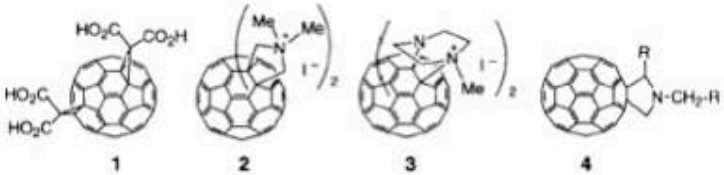












Impact of Surface Chemistry on In Vitro Cytotoxicity

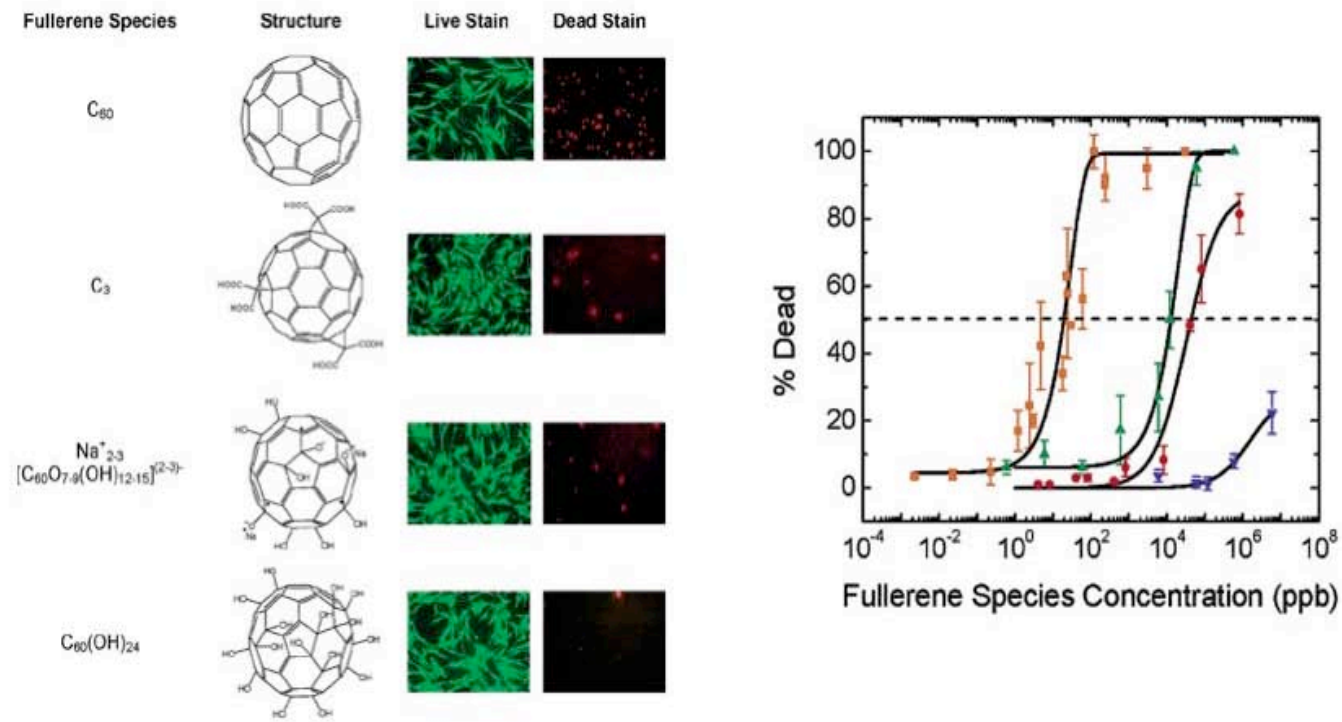
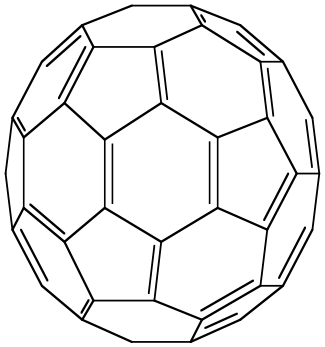


Figure 1. Differences in the structure and cellular activity of nano-C_{60}, C_3, $Na^+_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]^{(2-3)-}$, and $C_{60}(OH)_{24}$. The structure of each fullerene species is shown in the table, as well as the live and dead stains. (Bottom) The differential cytotoxicity of nano-C_{60} (■) as compared to C_3 (▲), $Na^+_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]^{(2-3)-}$ (●), and $C_{60}(OH)_{24}$ (▼) in human dermal fibroblasts. Cells were exposed to toxicant for 48 h.

Sayes et al 2004, Nanoletters 4:1881-1887

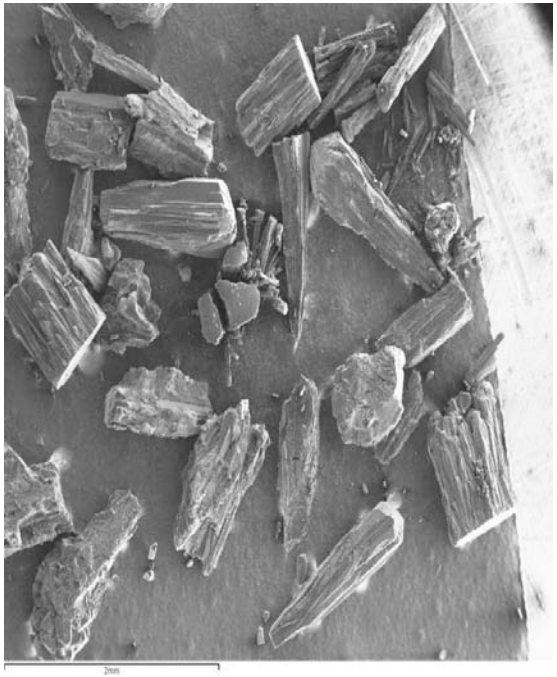
Fullerenes

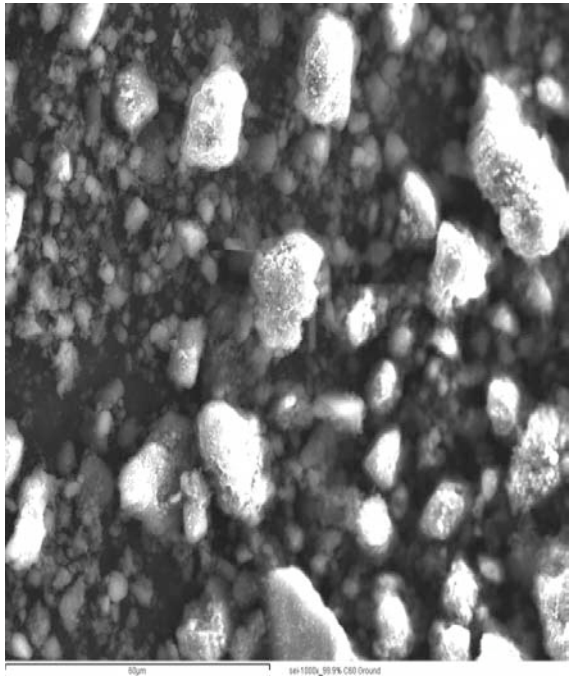
- Rationale:
  - Growing use and/or potential applications in microelectronics, batteries, fuel cells, polymers, composites and in drug delivery
  - Frontier Carbon- Japan ~40 tons/year- US production planned
  - What to study?
    - C60 99.9% (pristine)
    - Fullerene “soot” containing C60, C70 and higher order fullerenes
    - Hydroxylated fullerene
    - Hydrogenated fullerene
    - Nanocrystalline nC60-crystal composed of C60 and hydroxylated C60

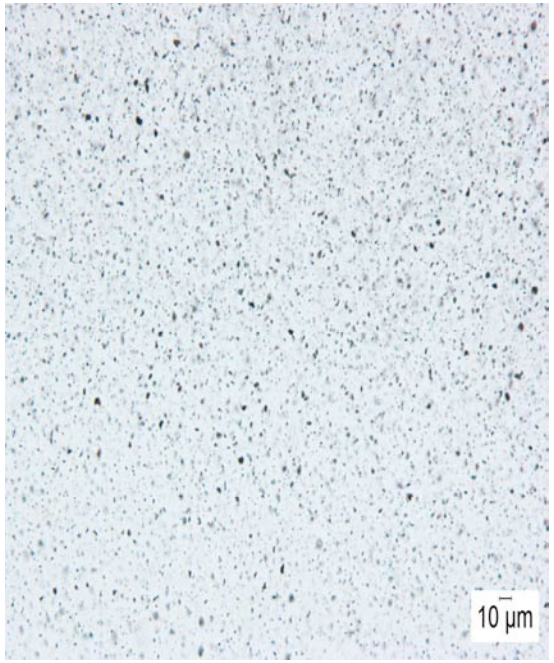


What to Study?

- Fullerenes (99.99% pure) tend to agglomerate
- Which form will humans encounter?
- Will they “dissolve” in human tissues/fluids?

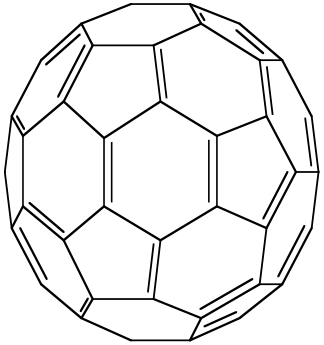






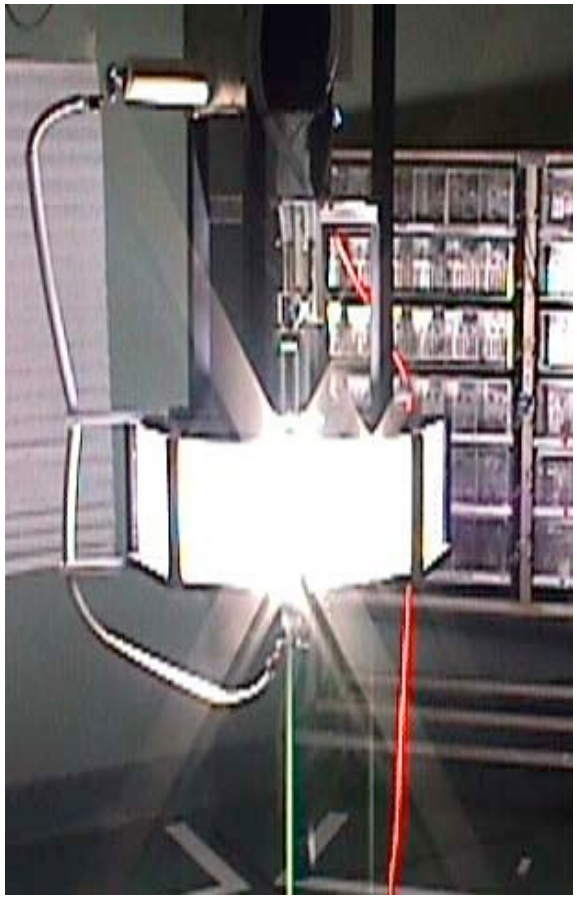
Fullerenes

- Ongoing NTP rodent toxicology studies
  - 2-week studies by oral, dermal and intratracheal instillation routes (various materials), inhalation (C60)
    - Endpoints- pulmonary inflammation, airway hyper-responsiveness
    - Immunological assessments
    - General toxicology assessment including histopathology, clinical pathology
    - Tissue accumulation, elimination kinetics
    - Genetic toxicity
    - Markers of reproductive and developmental effects
  - 90-day oral and inhalation toxicology studies (C60)



Dermal Uptake and Phototoxicity

- Interagency agreement - NIEHS and FDA
  - Utilize state of the art capabilities of the NTP Phototoxicology Center at the NCTR
- Focus on nanomaterials in cosmetics and sunscreens, use quantum dots as surrogates
- Study entry into skin and transport to other tissues from skin
- Study toxicity with and without exposure to simulated solar radiation



Understanding Surface Coatings is Key

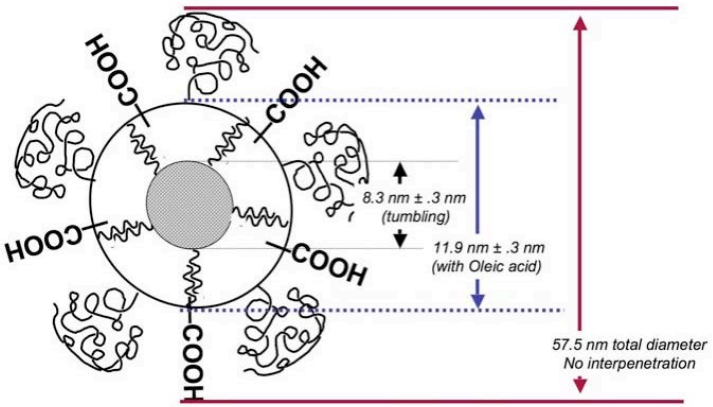
Coatings can:

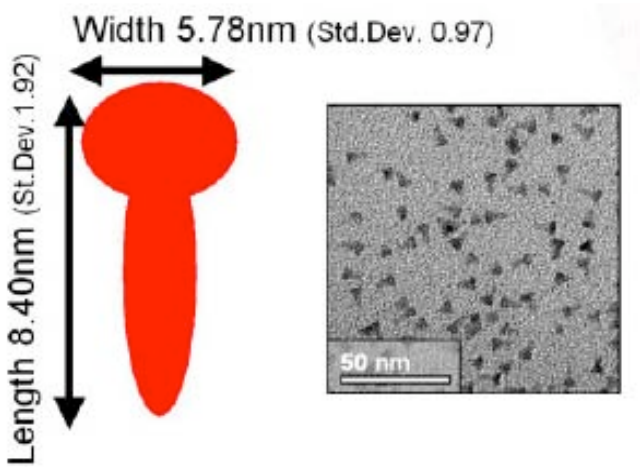
1) prevent agglomeration
2) encapsulate toxic metals
3) affect absorption and transport
4) prevent or cause immunological respose
5) modify or prevent toxicity
6) assist in elimination (presumably, not yet studied)

More complete knowledge of coating effects may vastly simplify the lives of toxicologists and regulators everywhere.

Quantum Dot Characterization

- CdSe/ZnS capped-PEG coated
  - Emission maxima; 621 nm or 655nm
  - 37 or 40nm size depending on method
  - Formulated in oil water emulsion
- Concentration
  - Absorbance spectroscopy
  - Cryogenic transmission electron microscopy (TEM)
  - Elemental analysis
- Hydrodynamic Size
  - Composition of the particles
  - Dynamic light scattering
  - Size exclusion chromatography

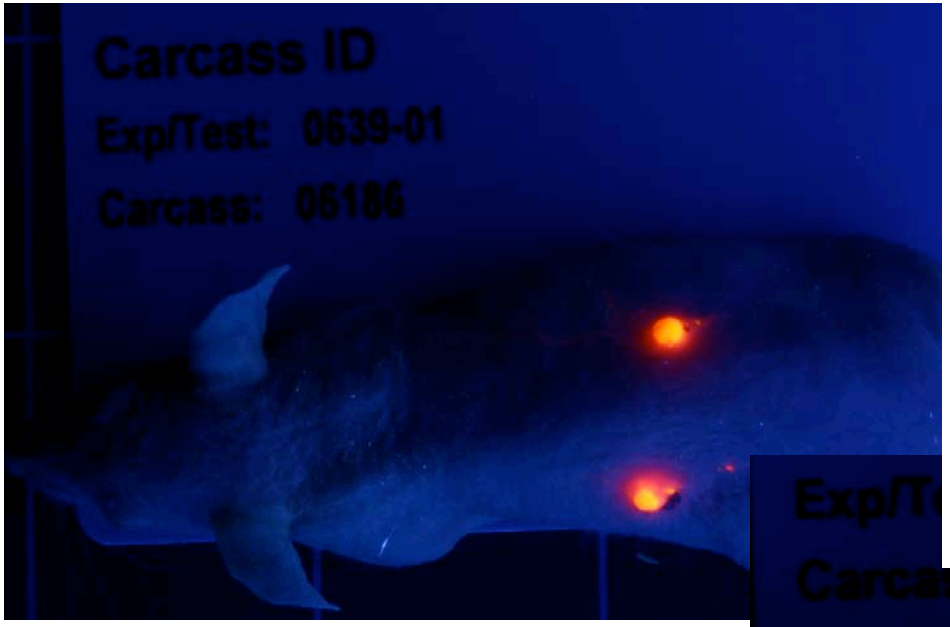


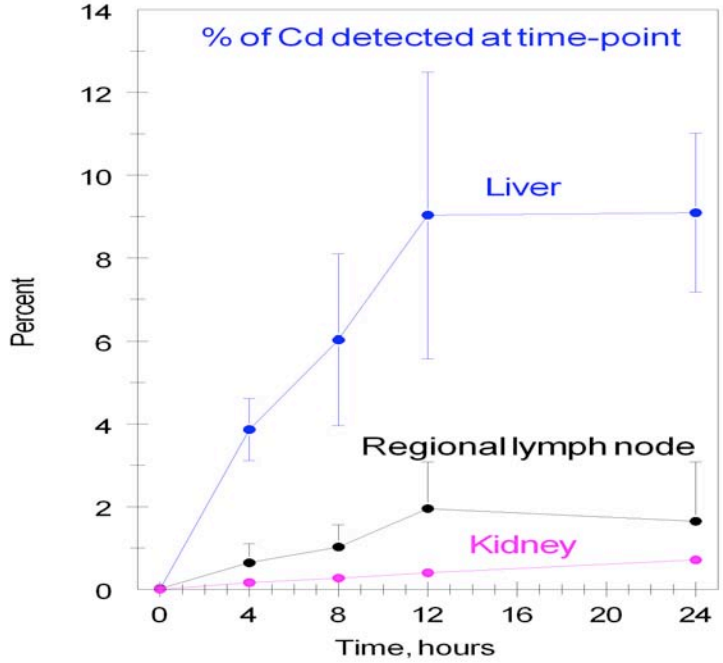


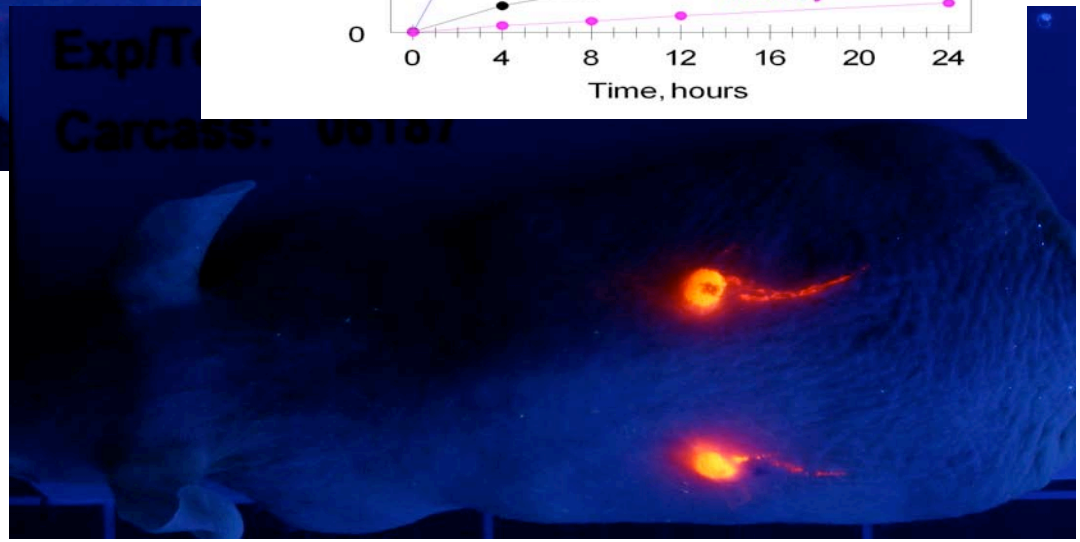
Dermal Uptake of Quantum Dots

- Q-dots, PEG coated, injected intradermally or applied topically to skin of SKH-1 hairless mice
  - Red Q-dots- 655 nm emission, 6 x 12 nm rods
  - Yellow Q-dots- 565 nm emission, 6 nm spheres
- Animals evaluated at time 0 and 6, 12, 18, 24, 48 hrs by
  - Photography using UV illumination
  - Fluorescent microscopy of formalin fixed tissue sections of site of application and draining lymph nodes

Intradermal Injection of Quantum Dots



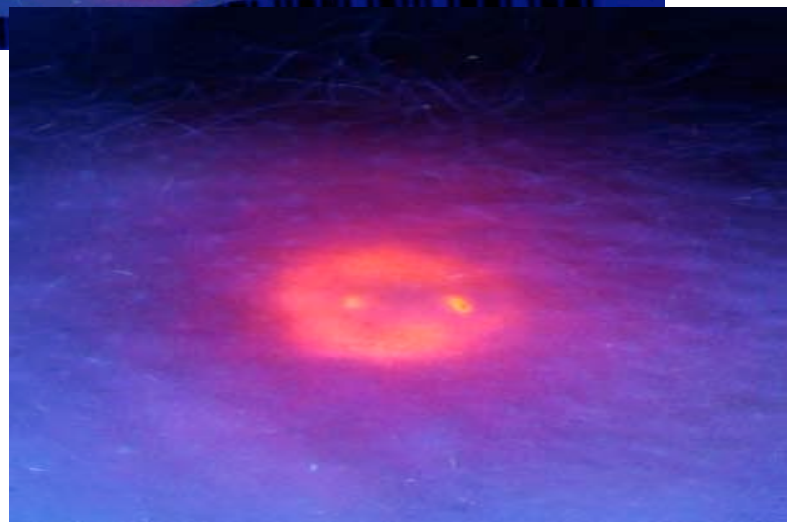




Intradermal Injection of Quantum Dots

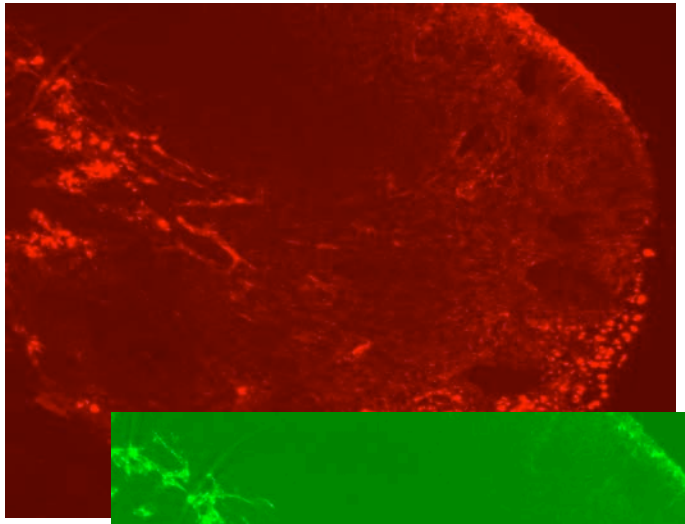
- Residual fluorescence at 6 hrs

- Residual fluorescence at 12 hrs

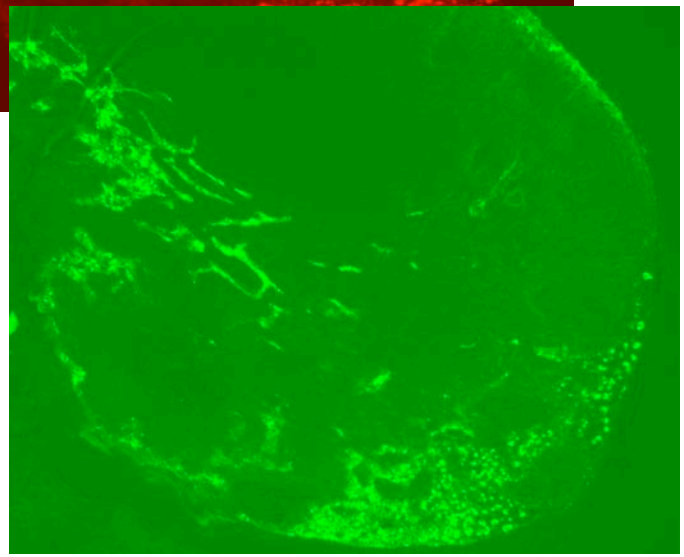


Intradermal Injection of Quantum Dots

- Axillary lymph node at 6 hrs - red Q-dots

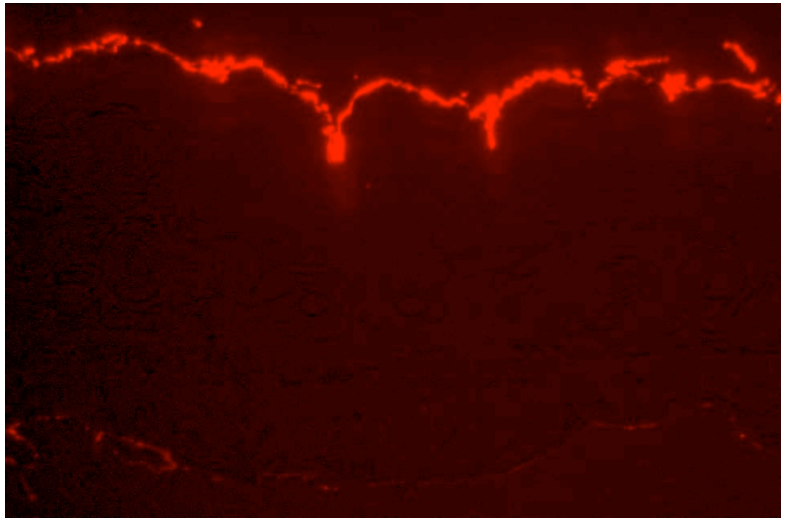


- Axillary lymph node at 6 hrs - yellow Q-dots

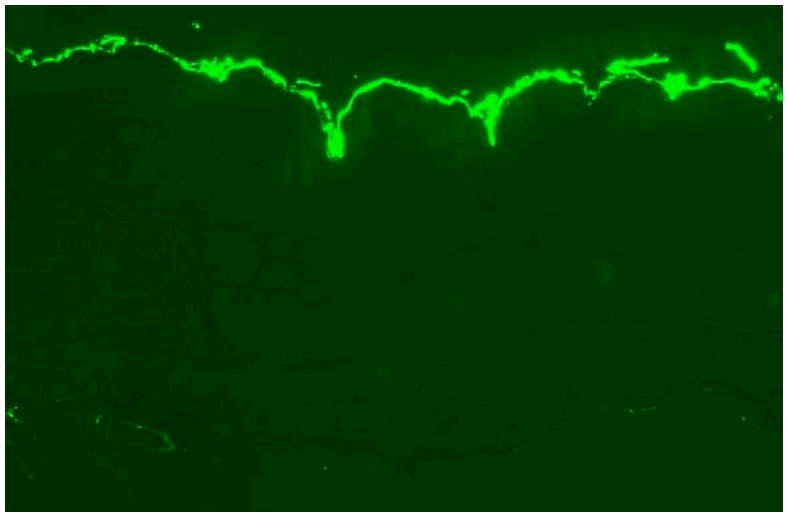


Topical Application of Quantum Dots

- Skin 0 hrs after topical application in oil/water emulsion, red Q-dots

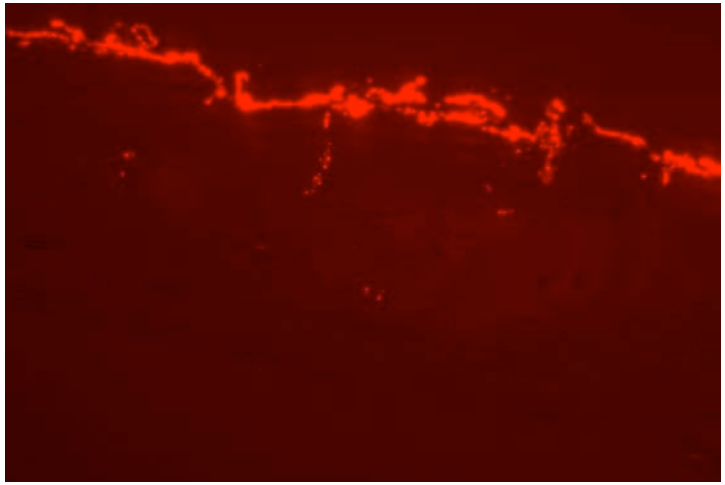


- Skin 0 hrs after topical application in oil/water emulsion- yellow Q-dots
  - Sections cut toward surface

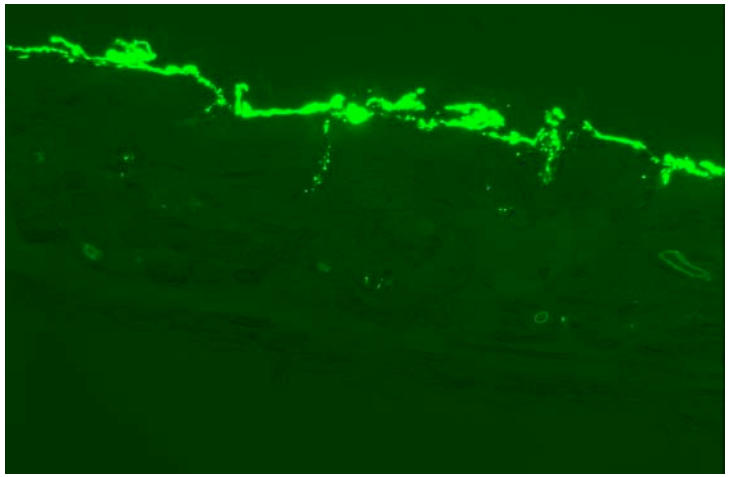


Topical Application of Quantum Dots

- Skin 18 hrs after topical application in oil/water emulsion, red Q-dots



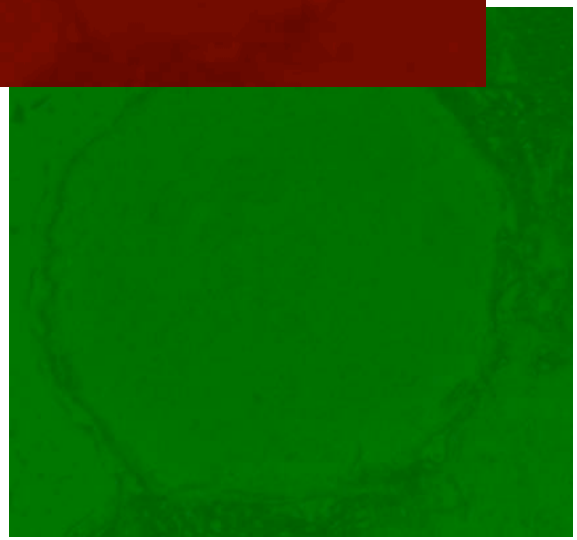
- Skin 18 hrs after topical application in oil/water emulsion- yellow Q-dots
  - Sections cut toward surface



Topical Application of Quantum Dots

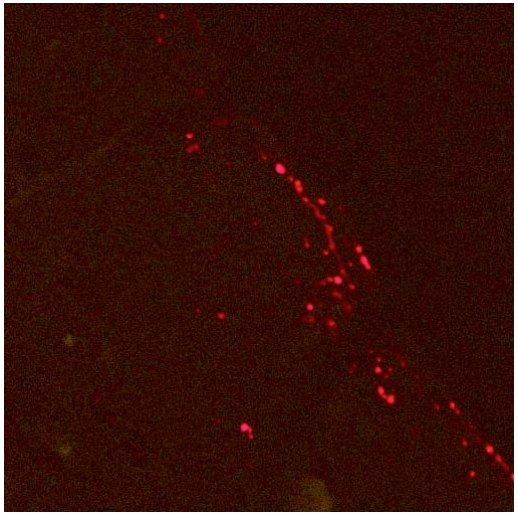
- Inguinal lymph nodes 48 hrs after topical application in oil/water emulsion, red Q-dots

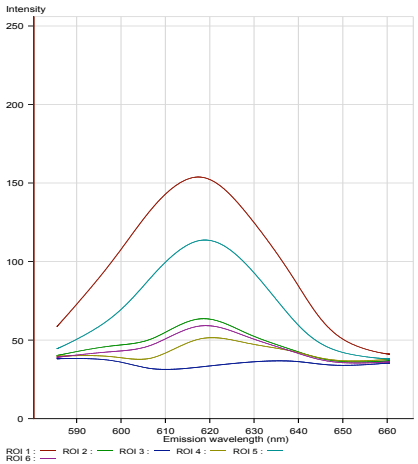
- Inguinal lymph nodes 48 hrs after topical application in oil/water emulsion- yellow Q-dots

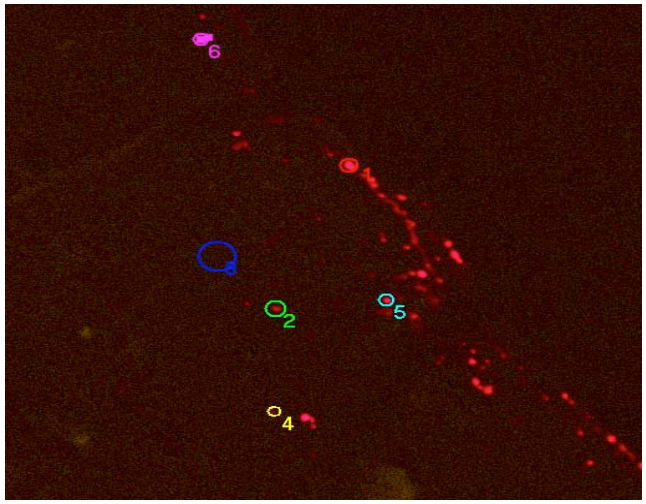


Examination by Laser Scanning Confocal Microscopy

- Internal fluorescence detected in Q-dot-treated skin
- Not all fluorescence is consistent with Q-dot spectrum



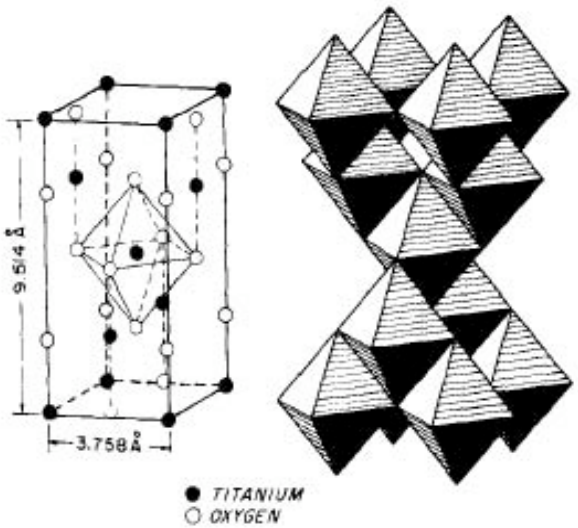




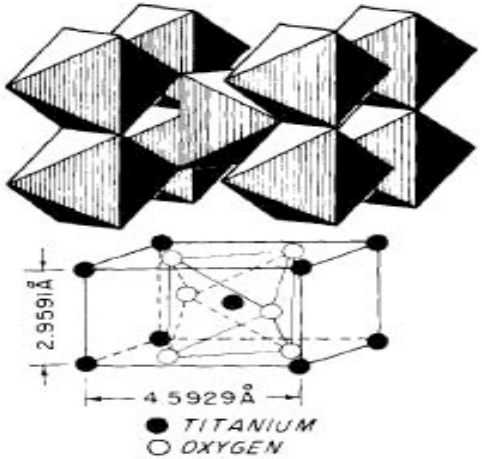
Titanium Dioxide in Sunscreens

- Predominant types of TiO2 in sunscreens- usually a mixture of:

Anatase- photoactive and a photocatalyst



Rutile- not photoactive



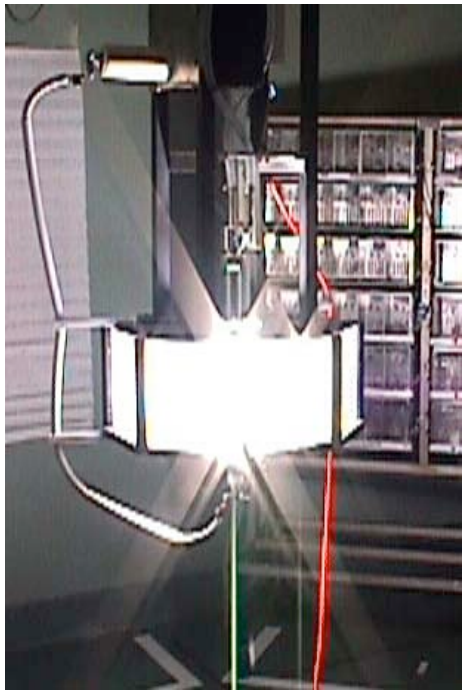
Images from: Millenium Chemicals Inc.
http://www.milleniumchem

Ongoing Studies of Metal Oxides in Sunscreens

- Center for Biological and Environmental Nanotechnology (CBEN) at Rice University

  Characterize sunscreen nanoscale oxides of titanium and zinc from 5 selected commercial products for:

  Size
  Crystallinity (TiO2 anatase or rutile)
  Morphology/aggregation state
  Elemental composition
  Surface coatings and surface area
  Generation of ROS



- NTP Phototoxicology Center, NCTR, FDA

  Evaluate potential for nanoscale Ti02 penetrate skin and to promote sunlight induced photocarcinogenicity in the SKH-1 hairless mouse

Impediments to Progress

- Specific nanomaterials with highest exposure potential not well known, thus difficult to Identify which materials are most important to study
- Characterization of materials more difficult than anticipated
  - Nomenclature not established
  - Poor communication between biologists, physicists and materials scientists
  - Analytical infrastructure not consistently available or well located
- Methods to detect nanomaterials in tissues or cells need further development
- Value of “in vitro” studies under debate
- Central repository of characterized nanomaterials not established
- Proprietary information developed by industry not available- may change

Bottom Line

- Should we panic?
  - No
- Is there reasonable cause for concern/caution?
  - Yes
- Is there a quick answer?
  - No
- Do we know what questions to ask?
  - Yes
- Are the right people asking the questions?
  - Yes
- Are we making progress?
  - Yes