Practical Nanotoxicology

David W. Hobson, Ph.D., DABT

LoneStar PharmTox LLC

Bergheim, Texas

dhobson@LoneStarPharmTox.com

Practical Nanotoxicology

- Introduction and Definitions
- Toxicology-Relevant Nanoproduct Concepts
- Nanotoxicology Fundamentals & State of the Science
- Regulatory Landscape
- Pharmaceutical & Medical Device Examples
- Acknowledgements
- Further Information
- Q & A

Introduction

- **What is "nano"**
  - **In general use it often is just anything smaller than "micro" (but not "atomic")**
  - **Technically, it is used when referring to a quantity of a substance or material that has at least one dimension between 1 to ≤ 100 nanometers whether naturally occurring, formed as a byproduct or created or "engineered" for some purpose**
  - **Practically, some have proposed that the nanoscale is from ≥ 1 nm up to ≤ 999 nm**
  - **Internationally it is viewed as the next big stage in the technological revolution beyond "micro"**
    - **"Microprocessors" may become "nanoprocessors"**
    - **Will Microsoft® become Nanosoft®?**

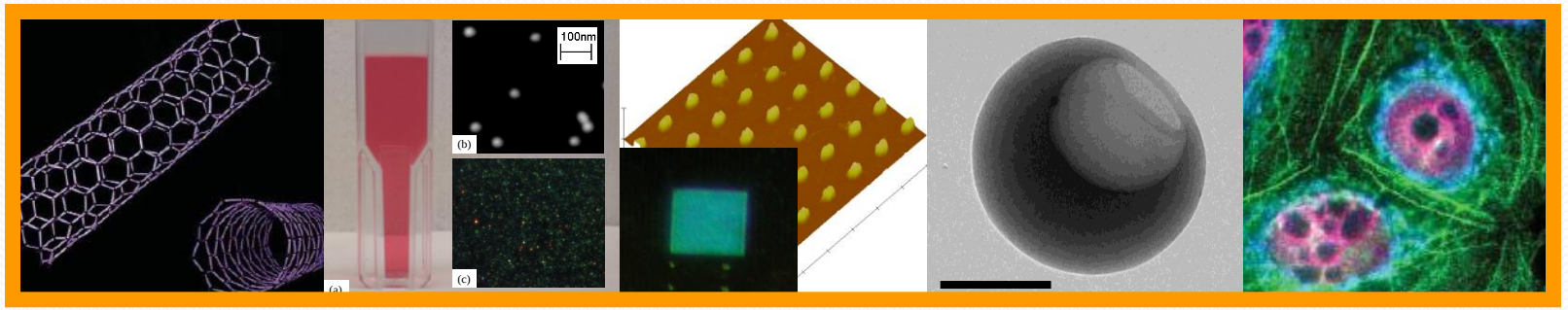
In the Universe, particles come in a wide range of sizes

Without light being scattered by nanoparticles sunrises and sunsets would not be as beautiful

Meteor Crater, AZ at 10,000 m – photo by DW Hobson

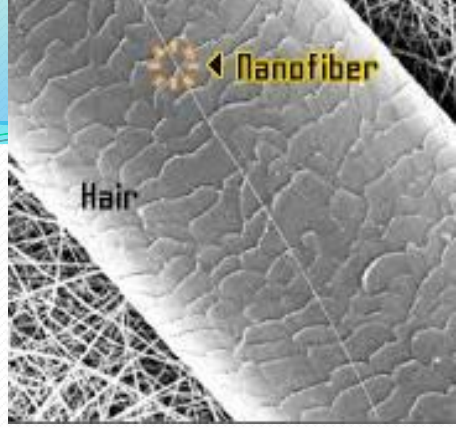
Nano – smaller and better

Nanoscale dimensions result in unique phenomena that enable novel applications



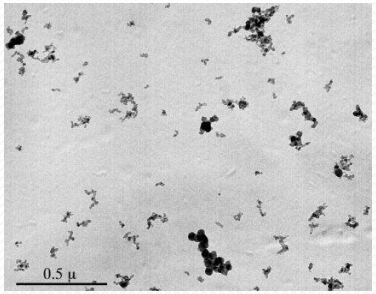
- Optical, electromagnetic, mechanical enhancement
- Increasing stability or reactivity, smaller size, higher surface/mass ratio

A new concept of small....



- As many nanoparticles fit into a grain of sand as grains of sand fit into a one-kilometer cube.
- At one hundred millionth of a meter. 10,000 would fit across a human hair.
- A tube of sunscreen can contain more nanoparticles of zinc oxide than there are grains of sand on a square kilometer of beach.
- Nanoparticles are larger than atoms but sometimes within the dimensions of the wavelengths of visible light and may exhibit different properties than the same material at larger dimensions.
- Gold (Au) which is typically shiny , yellow and metallic may turn purple at the nanoparticle scale, it's melting point may change and it can form crystals.

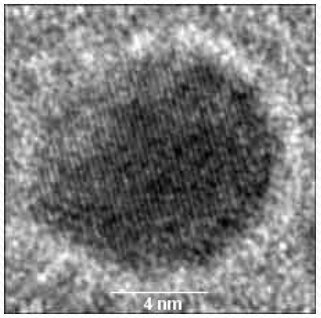
Nanomaterials are not exactly new….



- **Diesel exhaust emissions contain carbon / iron nanoparticles and agglomerates.**
  - Lee D, et al. (2005) J. Aerosol Science 37/1, 88-110

- **Ocean spray generates nanoparticles of various types.**

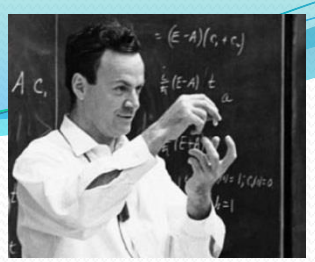
- **Natural precious and base metal nanoparticles can be found in sedimentary rocks, soil, sediment and atmospheric dust including volcanic ash.**



- **Nanominerals…. Seem to be everywhere!**

Nanotechnology

Still plenty of "room at the bottom"……



- **U.S. NNI** – "**Nanotechnology** - is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale."

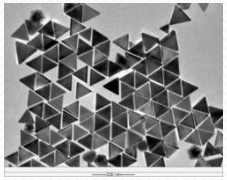
Nanotechnology

- **International Standards Organisation (ISO)** –
  - **Nanotechnology**: The application of scientific knowledge to manipulate and control matter in the nanoscale to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules or with bulk materials.
  - **Nanomaterial**: Materials with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.

- **Essentially, at this point, any feasible molecular structure to include any feasible nuclide can be constructed at will.**

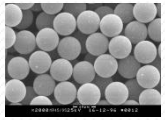
Useful Descriptors for Nanomaterial

nanoscale (Dimensional size range from approximately 1 nm to 100 nm)

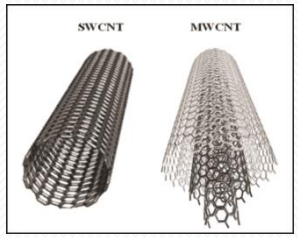


Silver nanoplatelets

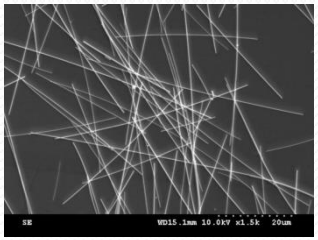
- **Nanomaterial**
  - **Nano-object** (any external dimension in the nanoscale)
    - **Nanoplate** (1 external dimensions in the nanoscale)
    - **Nanofiber** (2 external dimensions in the nanoscale)
      - **Nanotube** (hollow nanofiber)
        - **Single-Wall Nanotube** (hollow, single tube nanofiber)
        - **Multi-Wall Nanotube** (hollow, mutiple tube nanofiber)
      - **Nanowire** (electrically conducting nanofiber)
      - **Nanorod** (rigid nanofiber)
    - **Nanoparticle** (3 external dimensions in the nanoscale)
  - **Nanostructured material** (internal structure or surface structure in the nanoscale)



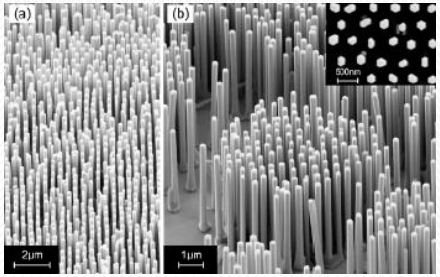
Gold Nanoparticles (100 nm)



Single- and Multi-Wall Carbon Nanotubes



Silver nanowire



Si nanorod arrays

Toxicology-Relevant Nanoproduct Landscape

- **More Concern**
  - Pharmaceuticals
  - Medical Devices
  - Cosmetics
  - Food and Feed
  - Industrial Chemicals
  - Agrochemicals
  - Emissions / Byproducts
  - … and more
- **Less Concern**
  - Semiconductors
  - Industrial catalysts
  - Polymers / Coatings
  - Fuels / Lubricants
  - Glass / Plastics
    - Permanently bound
    - Polymerized (non-degradable)

Nanotoxicology Fundamentals & State of the Science

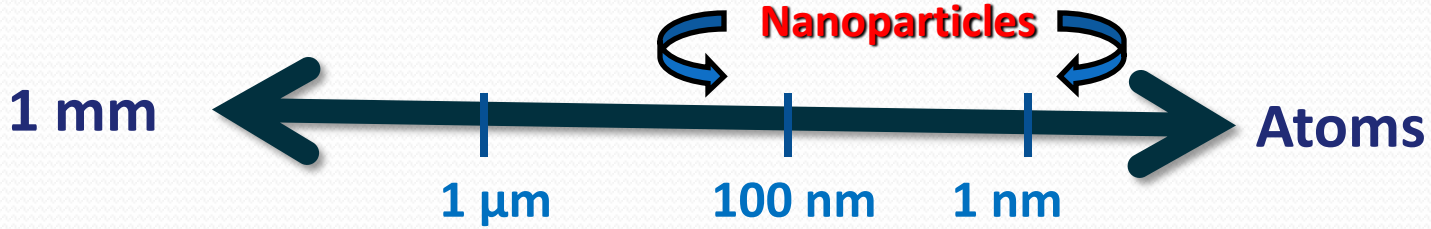
- Recognized in the 1990s that the same material in nanoparticle form can be more toxic than in the form of larger, still respirable, particles.
- "**Nanotoxicology**" – proposed by K Donaldson, V Stone, C L Tran, W Kreyling, P J A Borm (*Occup Environ Med* 2004;61:727–728.)
- Detailed mechanistic view in support of nanotoxicology proposed by G Oberdörster, E Oberdörster, J Oberdörster (*Environ Health Perspect.* 2005 July; 113(7): 823–839.)
- ***Why might nanoparticles be inherently more toxic than the substances of which they are composed?***

What is "nano?"

(a toxicologic perspective)

Macroscopic world:
"Classical Physics"

Nanoscopic world:
"Quantum Physics



Living Cells

Cellular Structures & Organelles

Biomolecules

Most Drugs

Nanotoxicology Fundamentals & State of the Science (cont.)

- **“Nano” size facilitates:**
  - Inhalation and gastrointestinal absorption
  - Uptake into cells and transcytosis across cells
  - Distribution into the blood and lymph circulation to reach potentially sensitive target sites such as bone marrow, lymph nodes, spleen, liver, kidneys and heart.
  - Brain entry via nasal nerves (e.g. polio virus)
  - Recognition and processing by the immune system
  - Entry into the cell nucleus
  - … and other toxicologically significant processes

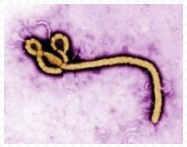
A Practical Perspective of Nanoparticle Hazard

- **“We don’t know anything about the risks associated with nanoparticles.”**
- **Actually… we do know something of nanoparticle safety.**
  - Particles that are relatively safe – **Low Hazard**
  - Particles that are known very harmful – **High Hazard**

Low → High

Engineered Nanoparticles ?

Natural Aerosols



Viral Pathogens

Nanotoxicology Fundamentals & State of the Science (cont.)

- Can nanoparticles (and nanomaterials) be engineered to be safe by design?

- What are the essential characteristics that make one nanomaterial more or less toxic than another?
  - Composition, size, shape, surface characteristics, aggregation / agglomeration state, molecular structure, etc.

- How should we express exposure / dose for nanomaterials? (*Teeguarden JG et al., Toxicological Sciences,* 95(2), 300–312, 2007)
  - *In vitro*
  - *In vivo*

Nanotoxicology Fundamentals & State of the Science (cont.)

Significant Issues

- "nanomaterials cannot be treated in the same manner as chemical compounds with regards to their safety assessment, as their unique physico-chemical properties are also responsible for unexpected interactions with experimental components that generate misleading data-sets." (Doak SH, et al. *Mutagenesis* vol. 24 no. 4 pp. 285–293, 2009)

- Most nanotoxicity studies (> 70%) are done *in vitro*, using a wide variety of models. Many of which have not been validated to *in vivo* toxicologic effects.

- There are very few "control" or "standard" reference nanomaterials

Nanotoxicology Fundamentals & State of the Science (cont.)

- **Understanding Nanotoxicity**
  - Absorption, distribution, toxicokinetics and metabolism *in vivo* must be studied and understood for different nanomaterials.
  - Targets and mechanism(s) of action must be identified *in vivo* and evaluated *in vitro*.
  - Routes and rates of elimination must be identified and characterized

- At the rate at which some new nanomaterials are being developed, collecting this information presents a road block and high throughput screening techniques are being proposed and developed.

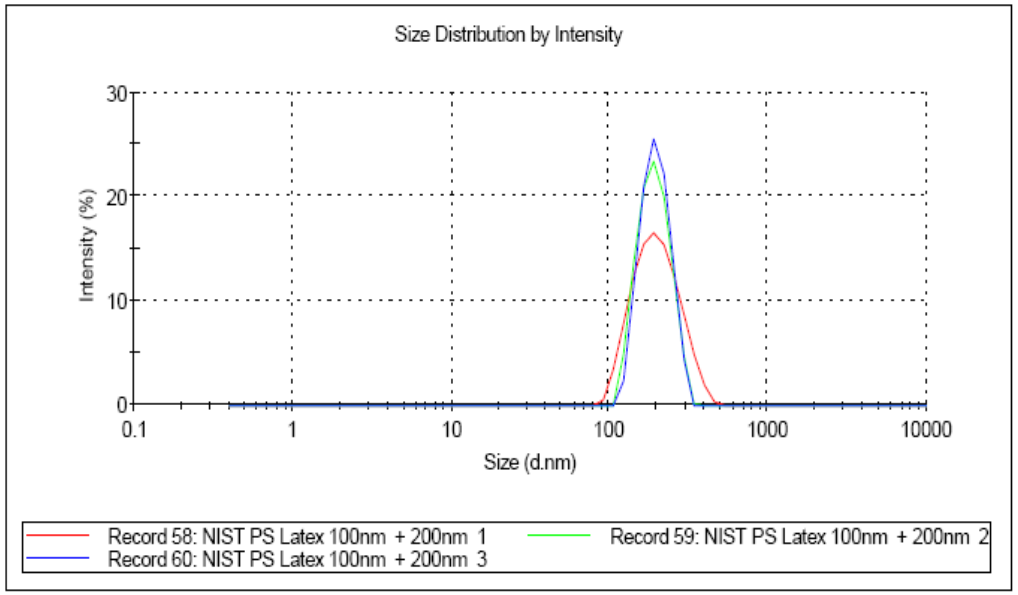
Typical Dynamic Light Scattering (DLS) Output

Mixed Sample of 100nm+200nm particles at a ratio of 10:1 by weight

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 190.4	Peak 1:	197.6	100.0	41.23
PdI: 0.008	Peak 2:	0.000	0.0	0.000
Intercept: 0.944	Peak 3:	0.000	0.0	0.000

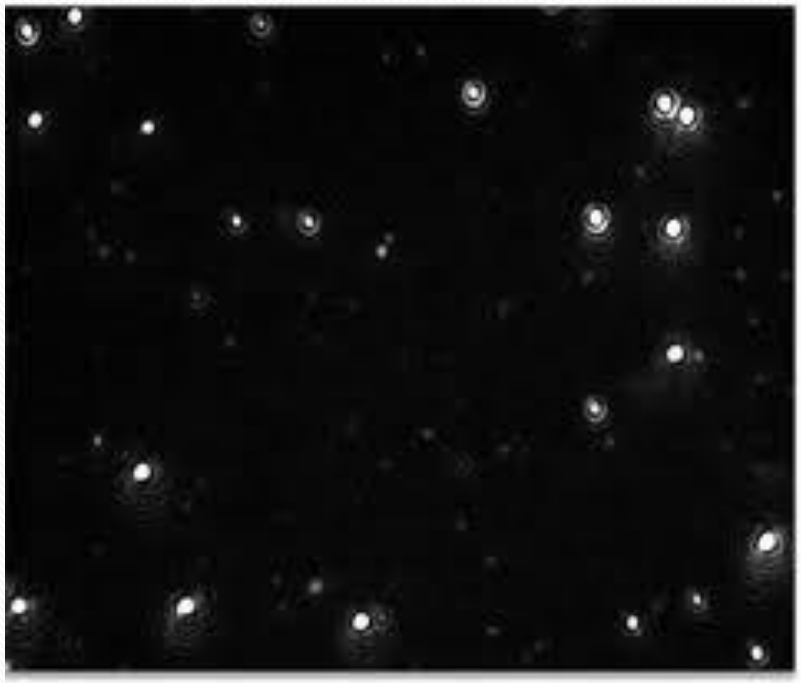
Result quality : Good

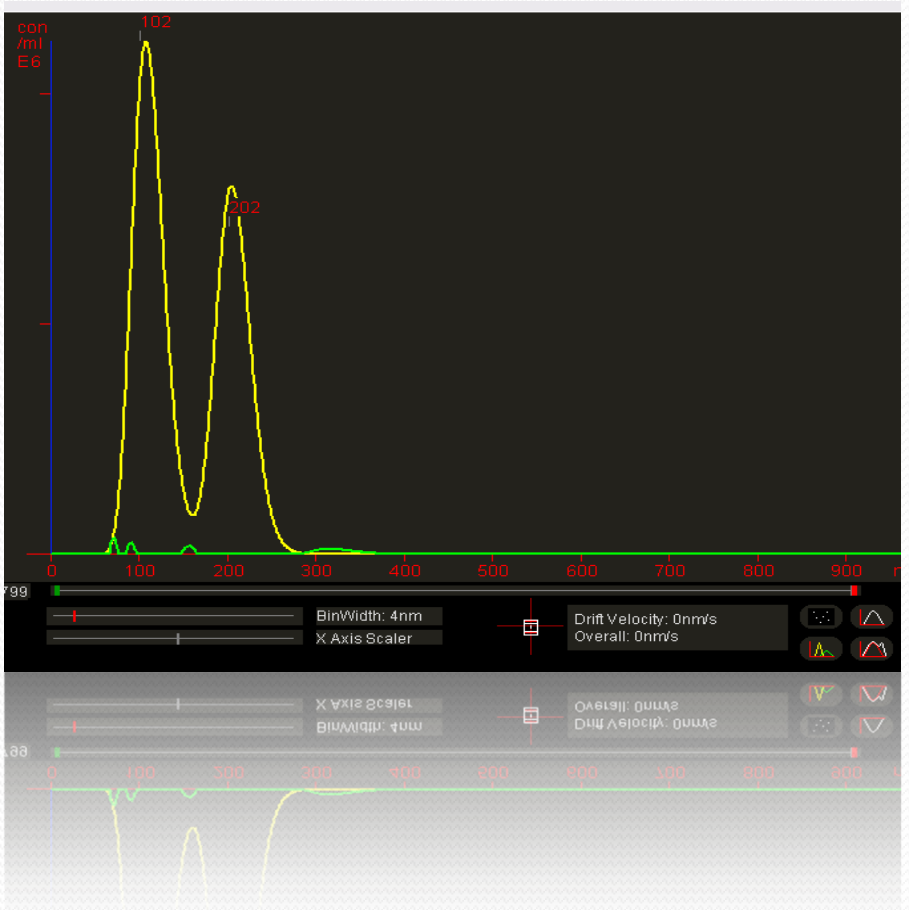


- **Rapid, repeatable result, but when polydisperse....**
- **....may miss complexity and be biased to larger particles**

Typical Nanoparticle Tracking Analysis (NTA) Output

Analysis shows both 100nm and 200nm peaks

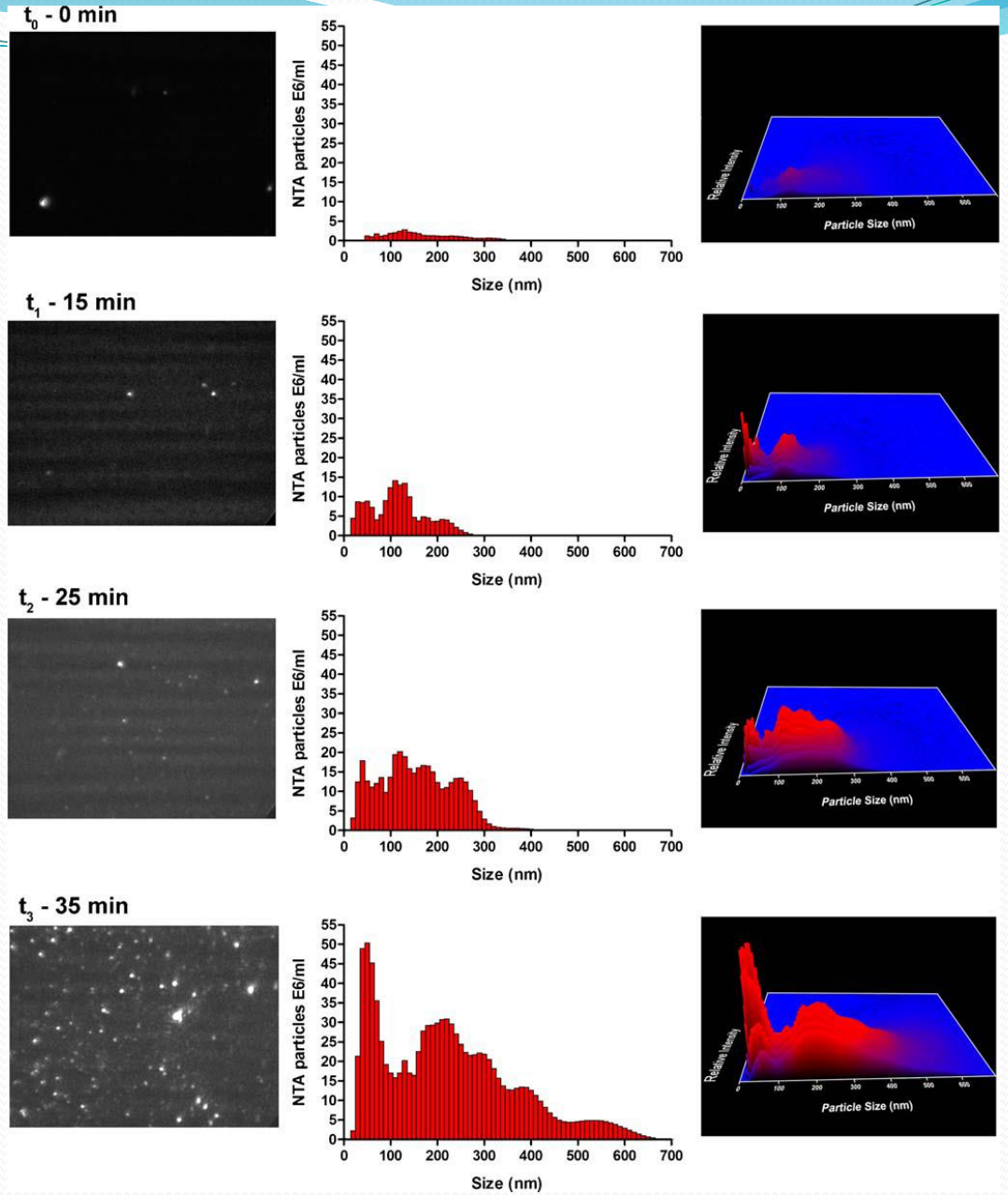




Polystyrene reference spheres in water (100nm and 200nm)

Typical NTA output

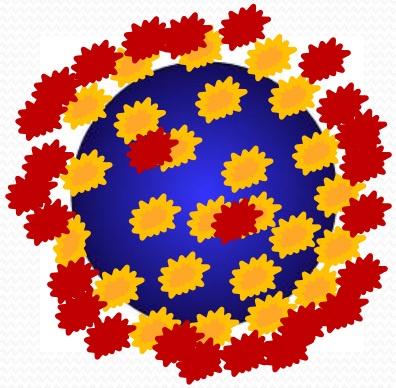
- Temperature-induced aggregation of IgG over 35 min.
- High-resolution particle size distribution
- Concentration measured, particle-by-particle
- Images visually confirm the data



Data reproduced from Filipe et al (2010), Pharmaceutical Research, DOI: 10.1007/s11095-010-0073-2

Nanoparticle Protein Corona Formation

- Particle size evaluation of nanoparticles in cell culture and *in vivo* may show formation of a protein "corona"
  - "Hard" – nanoparticle bound proteins ➡
  - "Soft" – proteins more weakly bound to hard corona proteins ➡
- Proteins may be nanoparticle unique and thousands of different types of proteins may be involved
- Immune system recognition, cellular processing, biodistribution, kinetics, elimination, etc.
- **May be pharmacologically and toxicologically very significant!**



Lundqvist M et al., Proc Natl Acad Sci USA 105:14265–14270, 2008

Aggarwal P et al., Adv Drug Deliv Rev 61:428–437, 2009

Karmali PP et al., Expert Opin Drug Deliv 8:343–357, 2011

Nanotoxicology Fundamentals & State of the Science (cont.)

- ***In vitro* study design considerations**
  - Nanomaterial and concentration (surface area exposure, aggregation / agglomeration state characterization, protein corona characterization, etc.).
  - Nanomaterial test system stability
  - Effect of nanomaterial on endpoint measurements

- ***In vivo* study design considerations**
  - **Similar as for in vitro above and…**
  - Nanomaterial and dose/exposure characterization
  - Nanomaterial ADME toxicokinetics
  - Normal endpoint monitoring (repro, cardio, pulmonary, behavior, body wt, etc.)
  - Histopathology (no nanomaterial-specific lesions as yet)
  - TEM / SEM localization of nanomaterial (if possible)

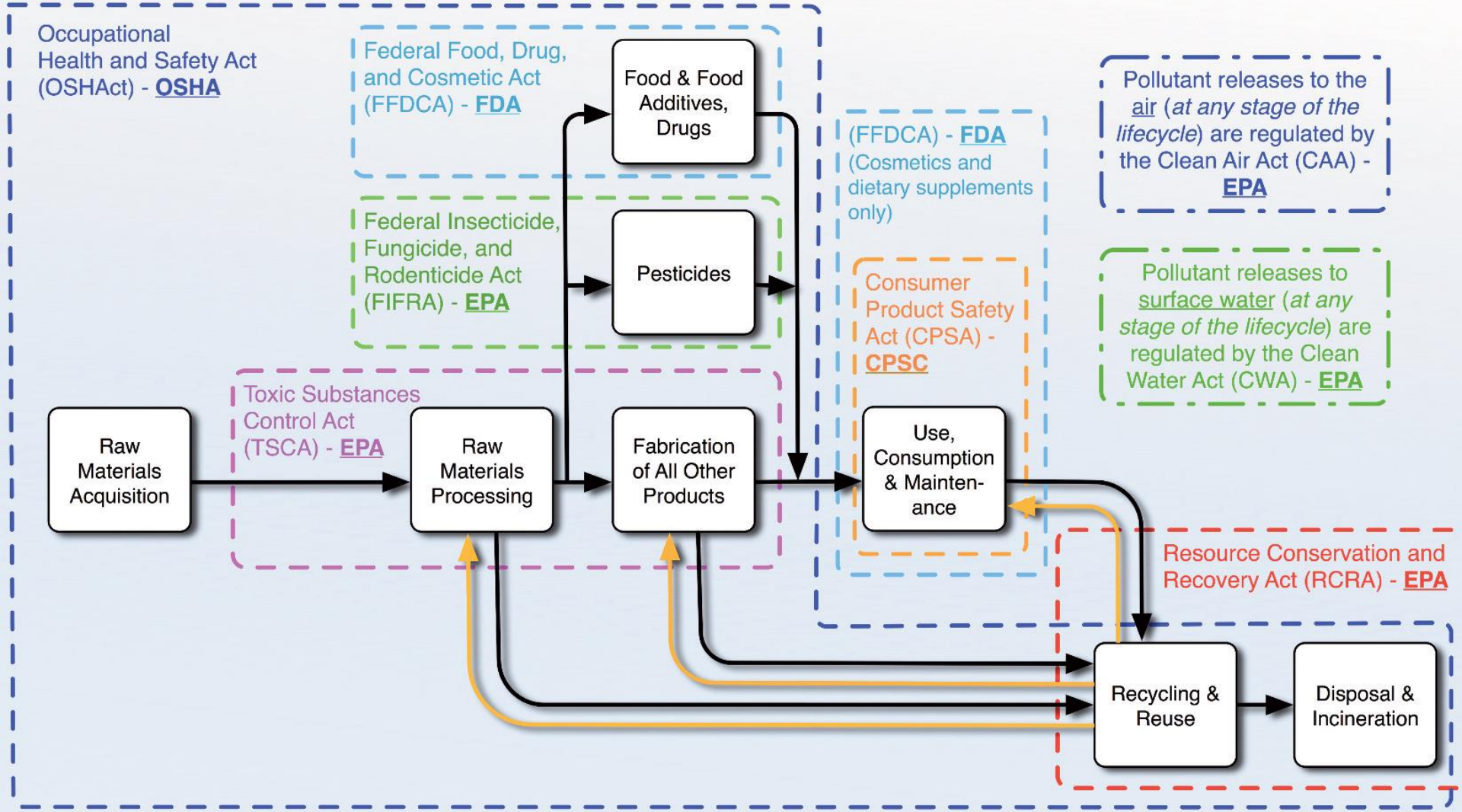
- **Publication of nanotoxicology study findings**

Safety Assessment of Nanoparticles

Practical Lessons Learned

- Dose expression needs to include particulate properties (dimension, shape, surface characteristics, charge, aggregation/agglomeration state, etc.) as well as chemical composition.
- Determination of biodistribution and kinetics while difficult, is very important. TEM is still the “gold standard” but new techniques (e.g. imaging) are emerging.
- The immunological recognition properties of nanoparticles can be significant and must not be overlooked.
- Whole animal perspective using *in vitro* methods to isolate and evaluate specific processes as necessary.
- Nondigestable particles sequestered in the RES (or some other tissue sites) may not have a route of elimination other than cremation!

U.S. Regulatory Landscape Comprehensive Over the Nanomaterial Lifecycle



Source: Chemical Heritage Foundation 2010

EU Regulation of Nanomaterials

- **Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)**
  - Substances, including substances at the nanoscale, manufactured or imported in volumes of 1 tonne or more per year have to be registered.
  - Nanomaterials are classified as hazardous under Regulation 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures must be classified and labelled.
  - European Commission envisages modifications may be necessary in some of the REACH Annexes.
  - Some NGOs advocate more stringent nanomaterial coverage
- **European Commission (EC) Directives** (Food, Pharmaceuticals, Cosmetics, etc.)
- **Individual Country Regulations**

The Regulatory Landscape : Are There Gaps?

- Some regulations may consider nanomaterials as “existing substances” or toxicologically equivalent to macrosized substances.
- Quantity thresholds that demand toxicologic (or ecotoxicologic) evaluation may be too high (in tons) as most nanomaterials are produced and used in small quantities (kilogram) but may disperse across a large product landscape.
- Nanomaterial definitions may be too vague. If it is > 100 nm, is it REALLY “nano?”

International Standards Organisation (ISO) - Nanotechnology

- ISO – TC 229 (established 2005)
  - Participating countries: 34
  - Observing countries: 12
  - Twenty seven (27) published ISO standards related to the TC and its SCs
  - All 27 published ISO standards under the direct responsibility of TC 229
    - http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=381983&published=on&includesc=true

ISO TC 229 – Toxicology Related Standards

- **Toxicology Specific**
  - ISO 10801:2010 - Nanotechnologies -- Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method
  - ISO 10808:2010 - Nanotechnologies -- Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing
  - ISO/TR 13014:2012 - Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment
  - ISO/TR 13121:2011 - Nanotechnologies -- Nanomaterial risk evaluation

- **Others of Interest**
  - ISO/TS 80004-1:2010 - Nanotechnologies -- Vocabulary -- Part 1: Core terms
  - ISO/TS 80004-5:2011- Nanotechnologies -- Vocabulary -- Part 5: Nano/bio interface
  - ISO/TS 80004-7:2011 - Nanotechnologies -- Vocabulary -- Part 7: Diagnostics and therapeutics for healthcare
  - ISO/TR 12885:2008 - Nanotechnologies -- Health and safety practices in occupational settings relevant to nanotechnologies

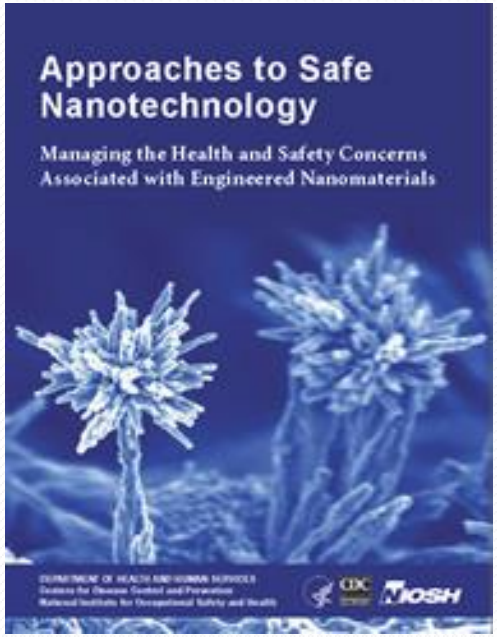
ISO/TR 13014: 2012 - Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment

- **Provides guidance for the physicochemical characterization of manufactured nano-objects prior to toxicological assessments.**
  - Physical character: What does it look like?
  - Constitution: What is it made of?
  - Interaction influences: How does it interact with the surrounding environment/media?
- **For *physical character*, the following parameters apply:**
  - Particle size/distribution;
  - Aggregation/Agglomeration state;
  - Shape: length to width with aspect ratio for fibres and elongated particles; and
  - Surface area/Specific surface area.
- **For *constitution,* the following parameters apply:**
  - Composition; and
  - Surface chemistry.

- **For *interaction influences*, the following parameters apply:**
  - Surface charge;
  - Solubility; and
  - Dispersibility.

Occupational and Environmental Safety for Nanomaterials

- **Excellent publications are available.**
  - DHHS (NIOSH) Publication Number 2009-125, **Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials**
- **Know your nanomaterial?**
  - **Toxicology data? MSDS?**
  - **Natural radionuclides, etc.?**
- **Absence of data ≠ No hazard!**
- **Use proper design controls & PPE**
- **Monitor exposure**
- **Check and monitor environmental release**



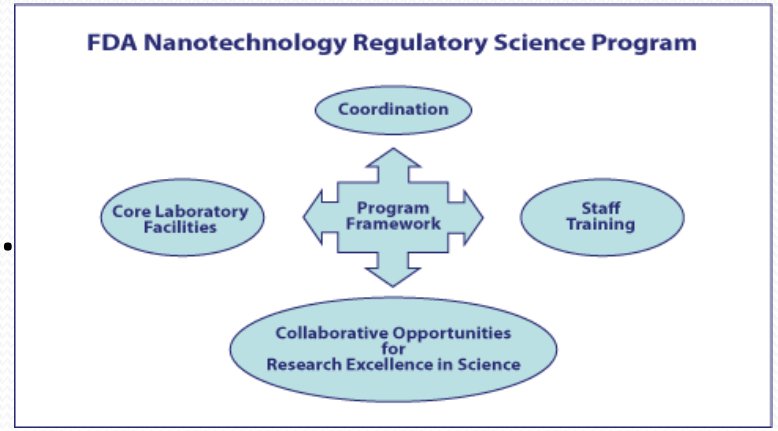
Perspectives on Nanotechnology Safety - FDA

- "Nanotechnology is an emerging technology that has the potential to be used in a broad array of FDA-regulated products, including medical products, foods and cosmetics."

- "These nanomaterials can have different chemical, physical, or biological properties than their conventionally-scaled counterpart materials used in many products regulated by FDA. "

- U.S. FDA has successfully regulated products that contain engineered nanotechnology for more than two decades.

- Currently over 20 drug products have been approved since 1990 that contain or utilize nanotechnology for therapeutic effectiveness.



U.S. Regulations: Food and Drugs

- **U.S. Food and Drug Administration (FDA)**
  - Nano foods and dietary supplements
  - Pharmaceuticals with nanoproduct APIs
  - Pharmaceuticals with nanoproduct “excipients”
  - Pharmaceutical and food product packaging
  - Cosmetics with nanoproducts
  - Medical devices
  - Medical diagnostics (in vitro diagnostics increasing)
  - “Case by case” review to date is working well
  - **FDA staff are actively familiarizing with nanotechnology**
  - **Guidance documents for regulated entities with nanoproduct components have been drafted**

New Guidances for Nanomaterials

- **U.S. FDA**
  - **July 2007 – FDA Nanotechnology Task Force report issued**
    - http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/UCM2006659.htm
  - **June 2011 – Issued a Draft Guidance for Industry : Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology**
    - http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm
  - **April 2012 - Draft Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives**
    - http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm300661.htm
  - **April 2012 - Draft Guidance for Industry: Safety of Nanomaterials in Cosmetic Products**
    - http://www.fda.gov/Cosmetics/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ucm300886.htm

Cleared "Nano" Pharmaceuticals

Some Examples

- **Liposomals**
  - **Liposomal Amphoterecin B - Mycotic infection**
  - **Liposomal Daunorubicin (DaunoXome) – Kaposi Sarcoma**
  - **Cytarabine liposome injection (Depocyt) - Lymphomatous meningitis**
  - **Collagran™ MMP inhibiting – wound dressings**
  - **“Stealth” liposome doxorubicin - Kaposi Sarcoma**
  - **Doxil/Caelyx – Ovarian / Breast cancer**
  - **Verteporfin liposomal (Visudyne) - Wet macular degeneration**
- **Solid Polymeric**
  - **Carmustine (Gliadel®) - Glioblastoma multiforme**
  - **Abraxane (nanoparticles of paclitaxel-taxol) - Mamary câncer (metastitic)**
  - **TrivCor (nanoparticulate form) - high cholesterol treatment**
- **PEGylated**
  - **PEG-succinimidyl-L-asparaginase – Lymphloblastic leukemia**
  - **PEG-adenosine deaminase – Serius immunodeficiency**
  - **PEG-interferon -2a (Pegasyls) - Hepatitis C**
- **Nanocrystal**
  - **Emend nanocrystals – Nausea prevention in chemotherapy**
  - **Rapamune nanocrystal - Rejection prevention**

Studies to Assess Safety for Nanotechnology Drugs / Devices

- The stated study objectives, study design and intended use of your data are important!
- There are established guidelines regarding the conduct safety studies (GLP); these pertain to all drugs and devices (including nanomaterials).
- Nanomaterials require additional characterization demands that are not necessarily required for other materials: multiple methods, purity, stability.

Nanoparticulate Pharmaceutical Products in Development

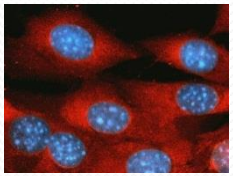
- There are nanotechnology-enabled products undergoing pre-clinical testing currently and a few in Phase 2 and 3 clinical trials at this time.

- These include many different forms of nanoparticles as well as a variety of indications. Newer forms have been “functionalized” in some way. Some are vectors for RNA

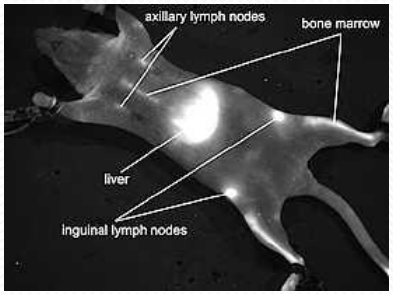
- In all instances safety evaluation is conducted on a “Case by Case” basis.

Typical IND/NDA Parameters for a Nanoparticle NME

- **Physical Characterization**:
- – Size
- – Size distribution
- – Molecular weight
- – Morphology
- – Surface area
- – Porosity
- – Solubility
- – Surface charge density
- – Purity
- – Sterility
- – Surface chemistry
- – Stability



- ***In Vitro***
- – Binding
- – Pharmacological effect
- – Blood contact properties
- – Cellular uptake
- – Cytotoxicity
- - Mutagenicity



- ***In Vivo***:
- – Absorption
- – Pharmacokinetics
- – Serum half-life
- – Protein binding
- – Tissue distribution
- – Metabolism
- – Excretion
- – Safety
  - For route and indication
  - Appropriate species
  - Appropriate duration
  - Relevant endpoints

Nanotechnology in Medical Devices: Considerations

- The primary toxicologic concern is generally for “unbound” nanomaterials
- Many devices may employ nanotechnology but may not require extensive safety evaluation if it can be established by design and relevant data that the nanomaterial is not bioavailable.
- *In vitro* diagnostic tests employing nanotechnology generally would not require *in vivo* safety or biocompatibility evaluation.
- The manufacturer is responsible for demonstrating a lack of potential exposure!

Nanotechnology in Medical Devices: U.S. FDA Draft Guidance

- **CDRH**:
  - **April 2013 – Draft Guidance for Industry: Use of International Standard ISO- 10993, "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing" (***not for implementation until final***)**
    - **Specifically addresses submicron or nanotechnology Components and indicates that considerations for dose characterization and the design and conduct of safety studies will have to take into consideration the unique properties of these materials. Requires understanding and application of current literature for accepted methods and procedures.**

Nanotechnology Regulation of Cosmetics

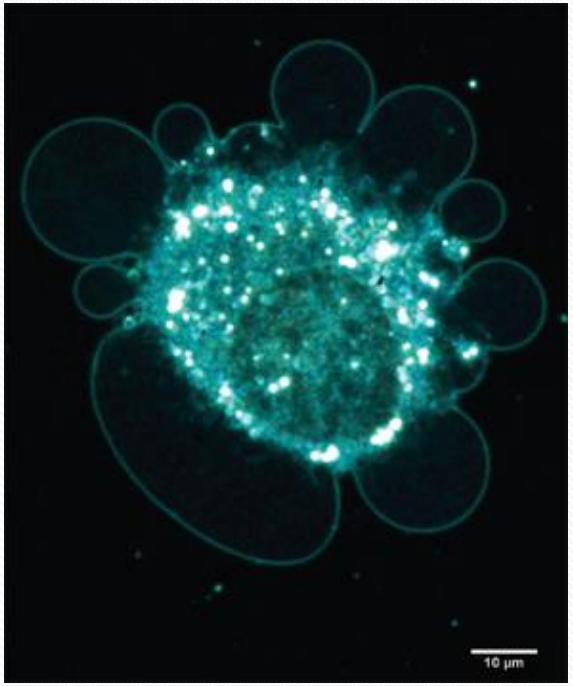
- **FDA draft guidance document.**
  - **Draft Guidance for Industry Safety of Nanomaterials in Cosmetic Products (April 2012)**: http://www.fda.gov/Cosmetics/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ucm300886.htm
- **"It is the responsibility of the manufacturer of a cosmetic product to ensure that the product is not misbranded or adulterated. Although the FD&C Act does not require the approval of FDA prior to marketing a cosmetic product, manufacturers or distributors should have obtained all data and information needed to substantiate the safety of the product before marketing."**

FDA Nanotechnology - Cosmetics

- **Important Considerations**
  - **For nanomaterials, manufacturers should consider modifying traditional toxicity testing with respect to such factors as appropriate solvents and dosing formulations, methods to prevent agglomeration of particles, purity and stability conditions, and other variables.**
  - **Dose characterization appropriate for nanomaterials should be completed and reported with each study.**

Example Nanotechnology Enabled Medical Products in Development

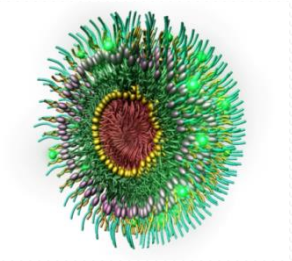
- **Cancer Chemotherapeutics**
  - Direct injection or selective uptake / molecular targeted localization into tumor tissue
  - **Metallic nanoparticles**
    - Radioisotopes, Au, Fe, In
  - **Cytotoxic, targeted nanoparticles**
    - Liposomes, PLGA nanospheres, etc.



Human ovarian cancer cell undergoing apoptosis resulting from internalization of a cytotoxic nanopharmaceutical (cytoxin nanoparticles).

From: D.W. Hobson, Pharm. Formul. & Quality, Feb/March 2010

Example Nanotechnology Enabled Medical Products in Development (cont.)

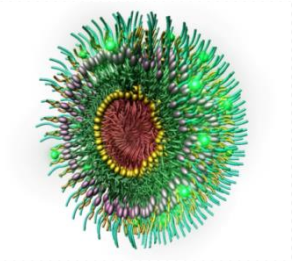


❑ HIV/AIDS Therapeutics

- APIs like azidothymidine (AZT) incorporated in PE-PEG coated solid-lipid nanoparticles (SLNs as a drug carrier can increase the bioavailability and gut reservoir of the incorporated API
- Pharmacokinetic behavior of the incorporated drug can be modified by changing the surface characteristics of SLNs by using amphiphilic solvation enhancers (Helati H, et al., *Int.J.Pharm*., 172,71-80, 1998).

- **A multitude of nanoparticle – API combinations are now being developed and tried.**

Example Nanotechnology Enabled Medical Products in Development (cont.)



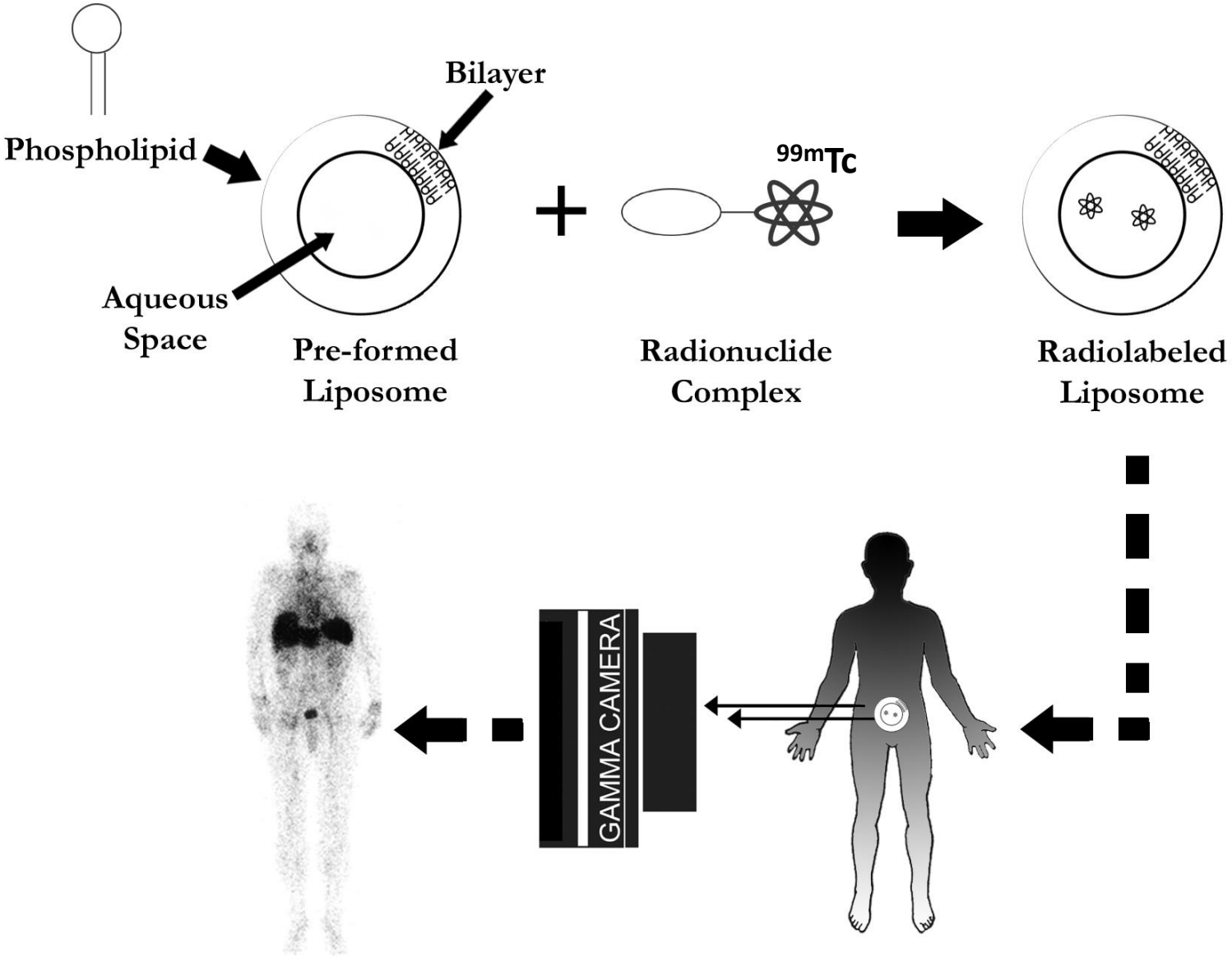
- **HIV/AIDS Therapeutics (cont.)**
  - Antiretroviral drug delivery with macrophage targeted peptide-PEG nanocarriers increased cellular uptake and increased accumulation in macrophages of liver, kidney and spleen compared with those which are non-targeted (Wan L et al., *Pharm Res.* Nov; 24(11):2110-9, 2007)
  - **Improved targeting increases antiretroviral concentrations where needed, with longer persistence and decreased systemic toxicity.**

Example Nanotechnology Enabled Medical Products in Development (cont.)

- **Nanoliposomal Therapeutics**
  - Low toxicity, metabolized and/or excreted via the lymphatics
  - Can be prepared for use with a wide variety of APIs
  - Various diagnostic radiolabels can be used to track nanometer-sized liposomes to monitor API delivery in the body quantitatively.
  - Radiolabeling techniques with a variety of common radiolabels for imaging (^{99m}Tc, ^{111}In, ^{67}Ga) have been developed for liposomes. (Phillips WT et al., *WIREs Nanomedicine and Nanobiotechnology*, Volume 1, January/February 2009)

Example: Bone Marrow Targeted (BMT) Liposomes (Sou K et al., *Expert Opin. Drug Deliv*. 8(3):317-328, 2011)

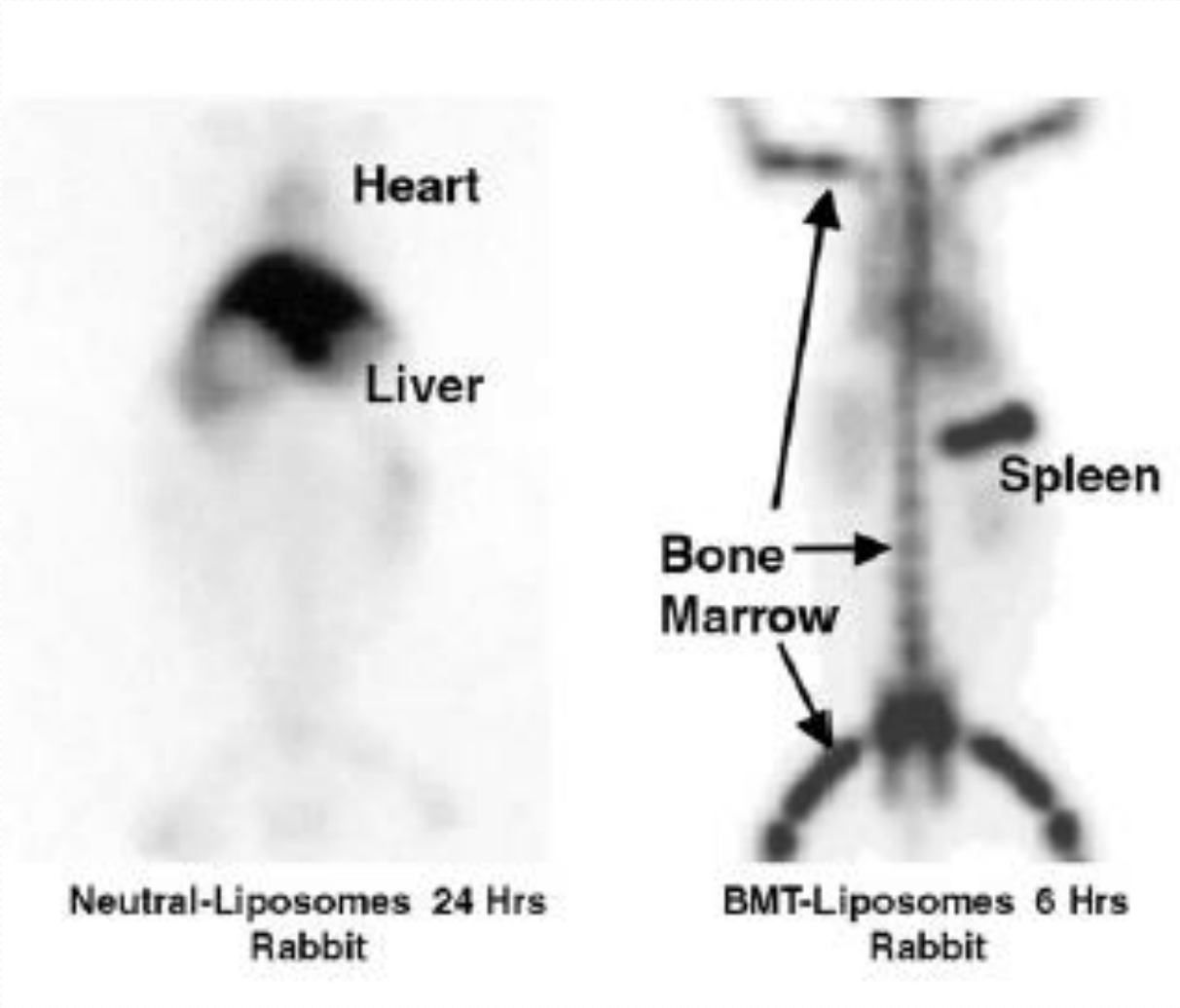
- **Four kinds of lipid** - 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC); cholesterol; L-glutamic acid; N-(3-carboxy-1-oxopropyl)-1,5-dihexadecyl ester (SA-lipid) and PEG
- **Delivery of agents that:**
  - protect the marrow from the toxic effects of chemotherapy and radiation,
  - ablate bone marrow effectively and safely before bone marrow transplant,
  - stimulate hematopoietic cell proliferation - new therapeutics for increasing hematopoiesis.


Phospholipid
Bilayer
Aqueous Space
Pre-formed Liposome
99mTc
Radionuclide Complex
Radiolabeled Liposome
GAMMA CAMERA

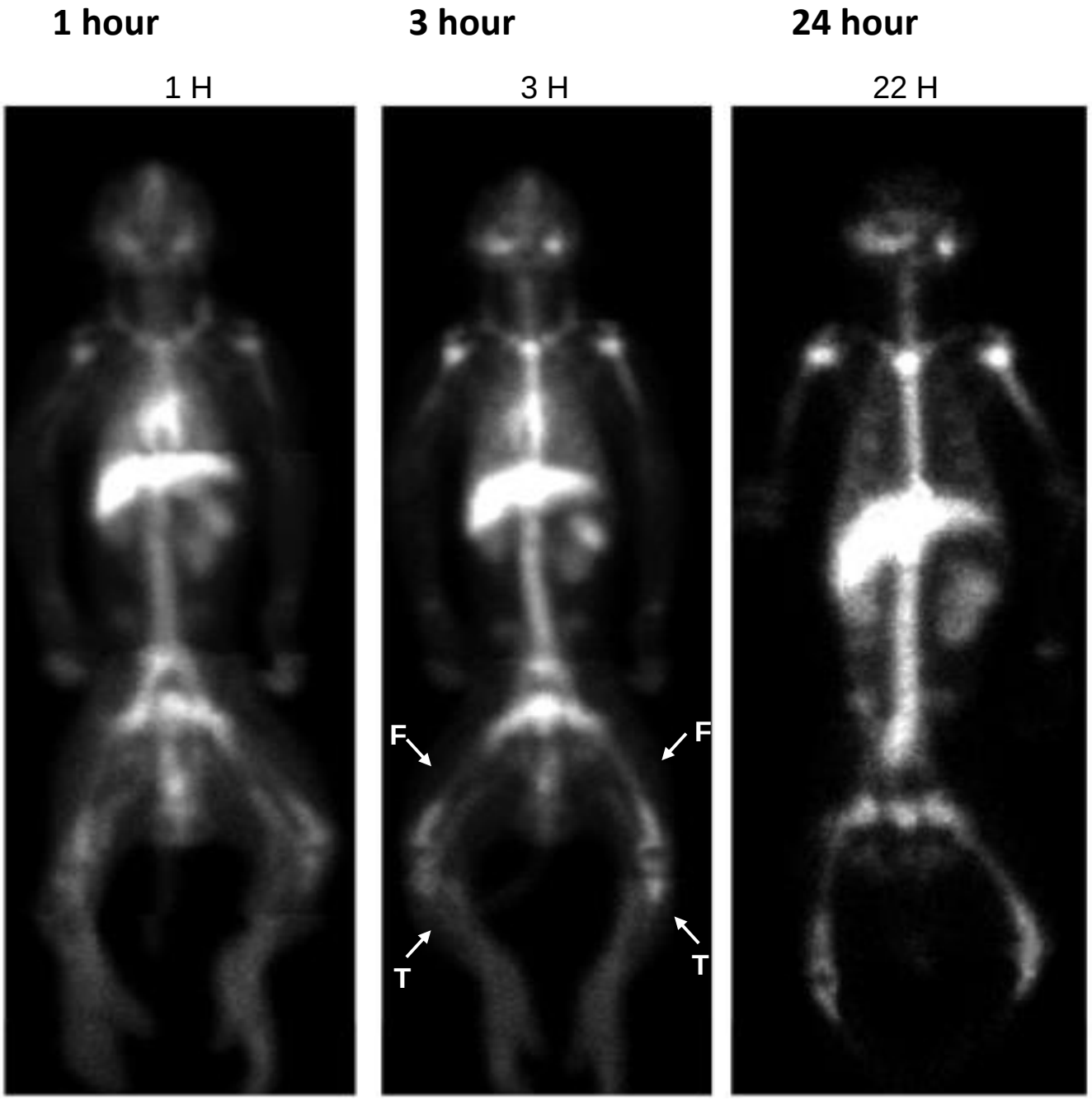
Example: Bone Marrow Targeted (BMT) Liposomes (cont.)

- Injected I.V. at 15 mg lipids/kg (body weight)
- rhesus monkey, rabbit, hamster, rat, mouse
- Whole body scintigraphic imaging
- Liposomal size range 200 -- 270 nm
- Radiolabel: ^{99m}Tc, photopeak of 140 keV and a half-life of 6 h
- Targeted areas of active myeloid hematopoiesis

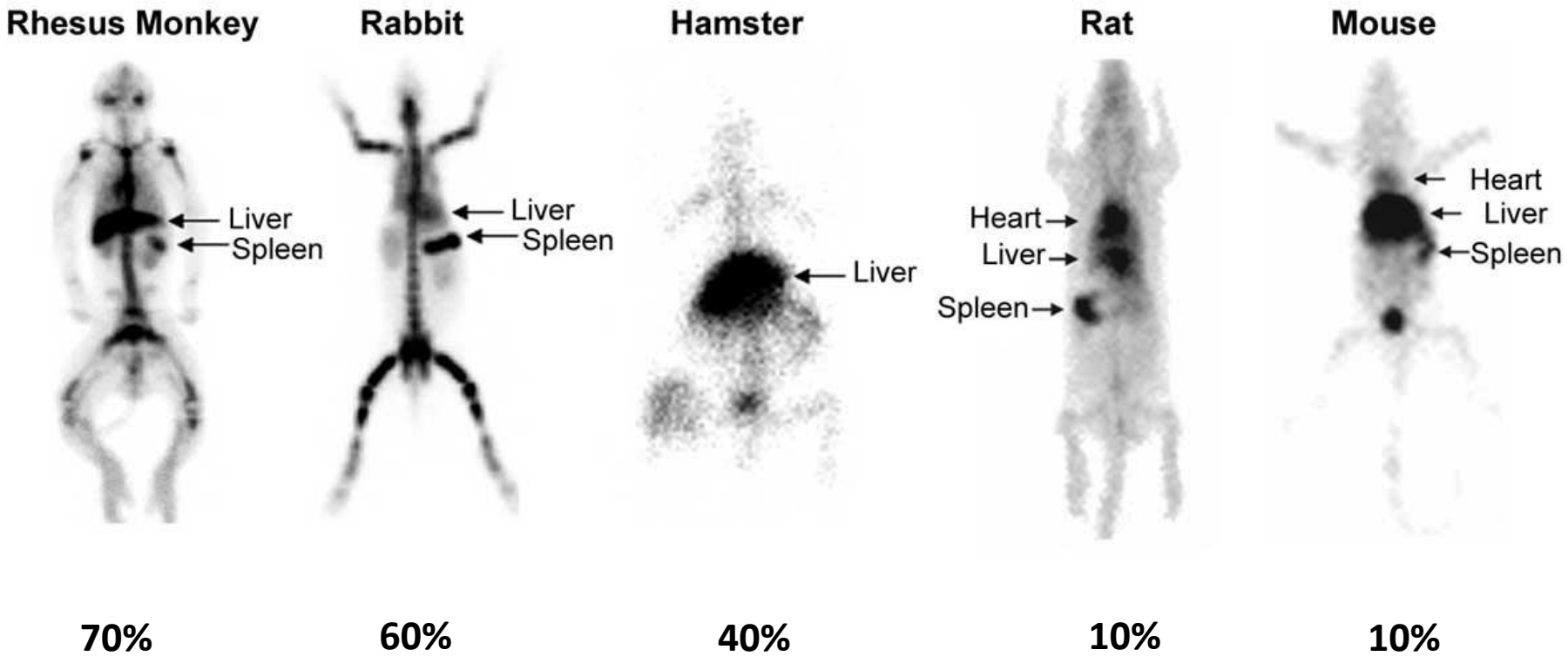
Bone marrow targeted liposomes versus controls



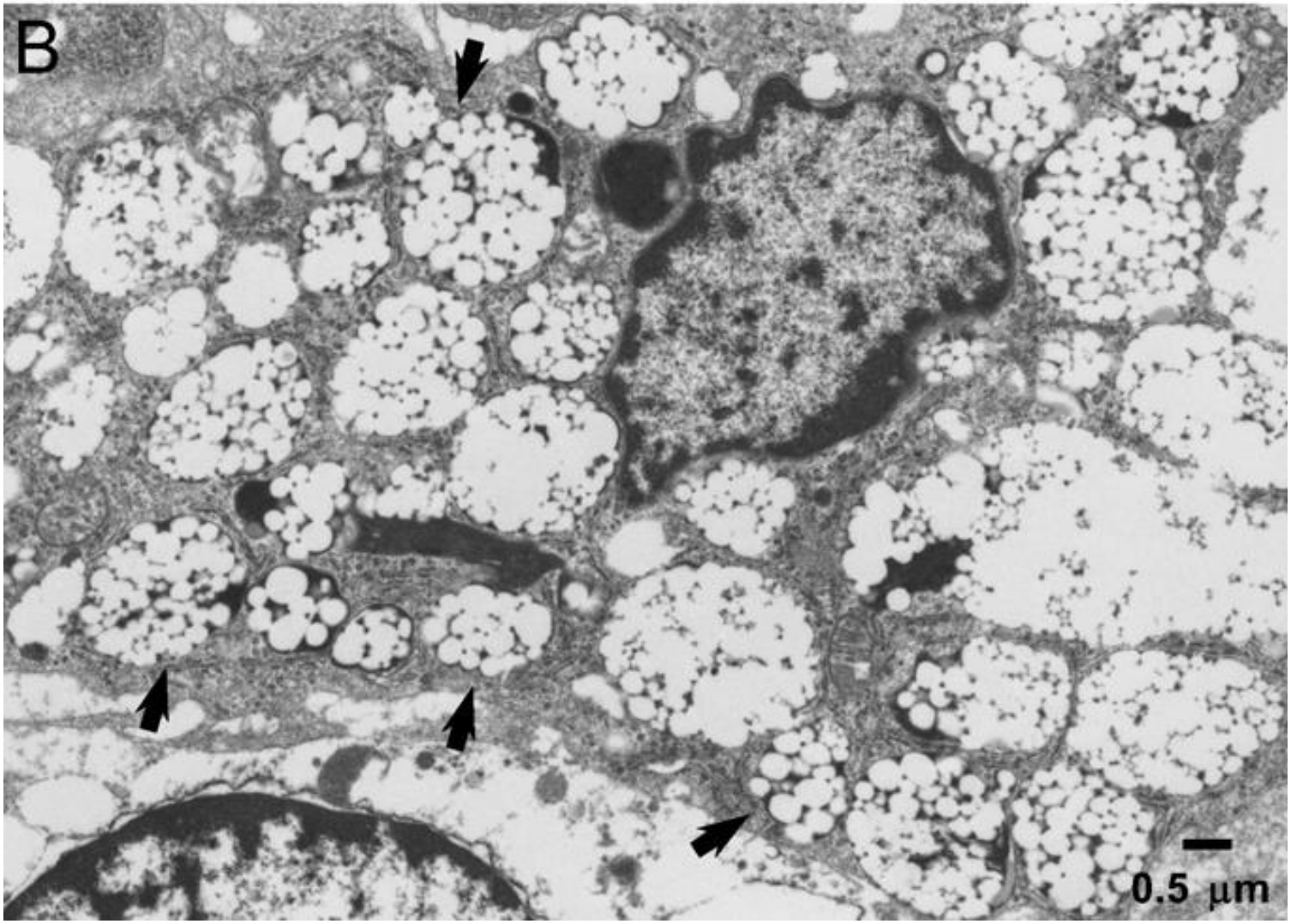
Rhesus Monkey



Species Comparison of Bone Marrow Targeted Liposomes

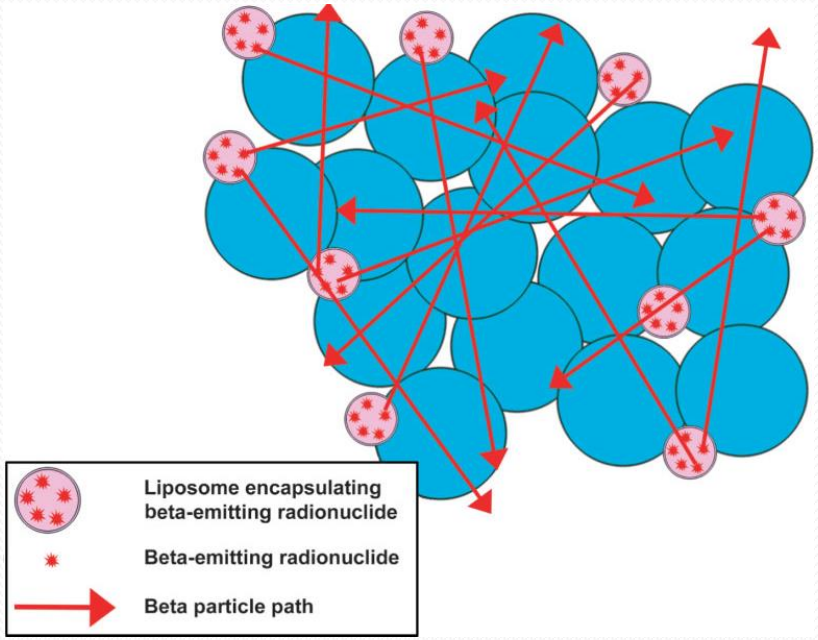


Bone Marrow Macrophage Liposome



Targeted Radiotherapeutic Liposomes

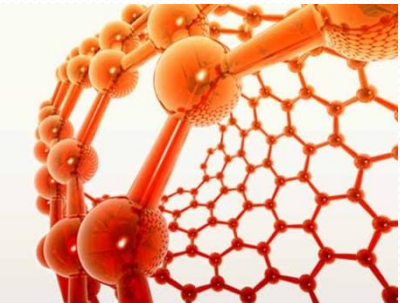
- Radiotherapeutic liposomes to treat residual tumor in the intraoperative situation.
- Injected in the region of the positive tumor margin to sterilize the surgical margin of tumor cells.



Phillips WT et al., *WIREs Nanomedicine and Nanobiotechnology*, Vol. 1, Jan/Feb 2009

Summary

- Nanotoxicology is an established subdiscipline
- Ever-increasing understanding of nanotoxicologic impacts as well as new product technologies and advancement of toxicological evaluation methods for nanomaterials
- New developments, advancements and opportunities may be expected for decades into the foreseeable future.



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Further Information

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