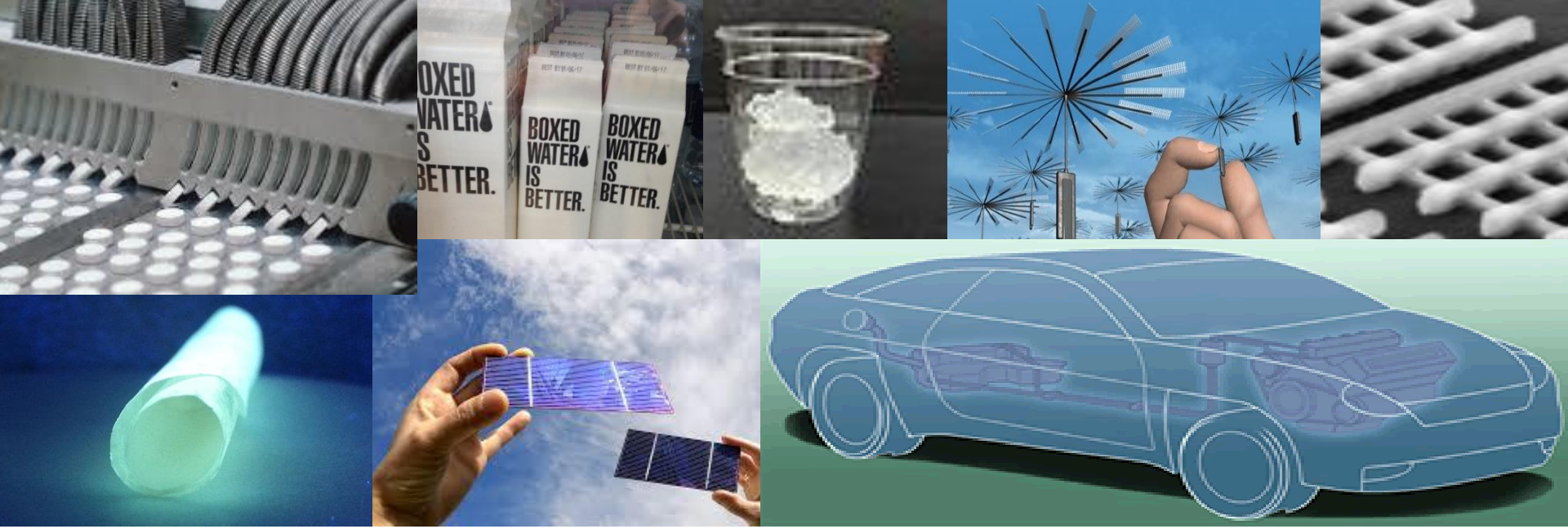

An Update on

Nanomaterial Toxicology, Exposure and Risk Analysis

Jo Anne Shatkin, Ph.D.

President, Vireo Advisors, LLC

Massachusetts TURA Science Advisory Board

November 30, 2016

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What We Do.

- We help our clients navigate the *regulatory landscape* for novel materials and technologies to get them to market
- We help our clients navigate the *environmental health and safety landscape* and manage safety aspects of new products and technologies
- We build and work with consortia to address *critical data gaps* necessary for commercialization

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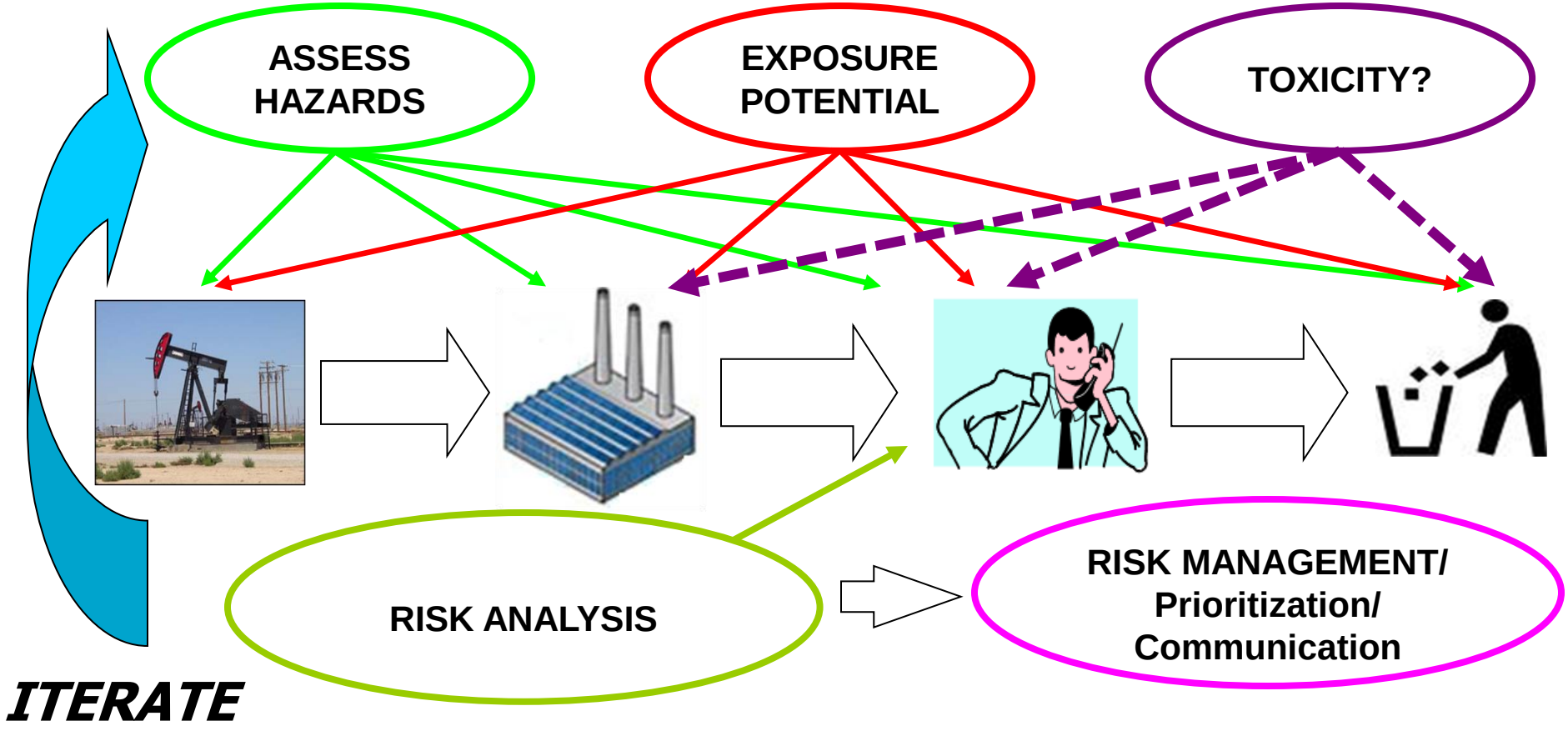
NANO LCRA: STREAMLINED LIFE CYCLE/RISK ASSESSMENT FRAMEWORK FOR NANOMATERIALS



Shatkin, J.A. (2012) *Nanotechnology Health and Environmental Risks Second Edition*. CRC Press.

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ISO Standard Definitions of "Nanoobjects"

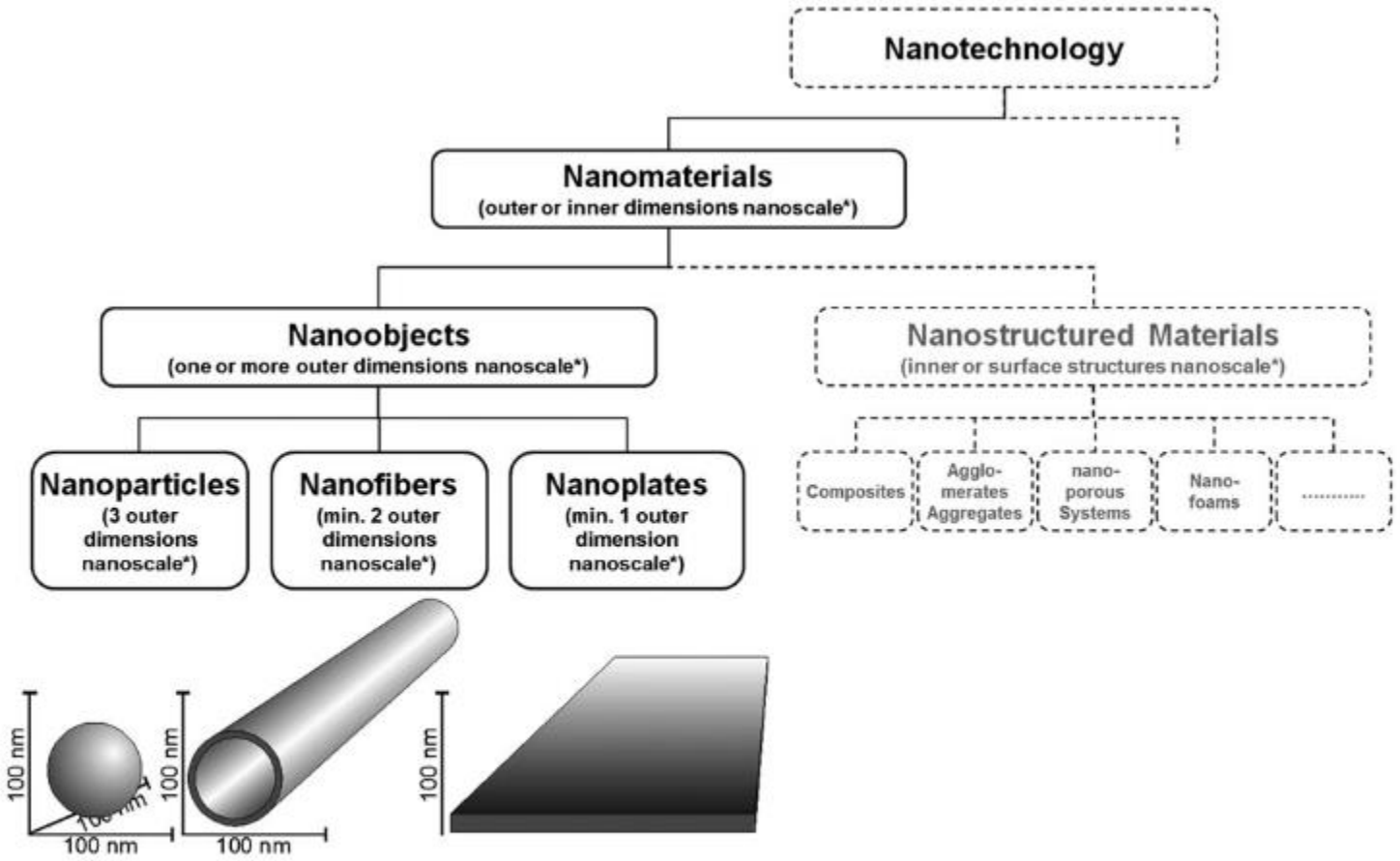

Figure 1. The ISO definition of nanoobjects. Included as nanoobjects are nanoparticles (nanoscale in all three dimensions), nanofibers (nanoscale in two dimensions), and nanoplates or nanolayers (nanoscale only in one dimension). * Nanoscale: a size of between 1 and 100 nm.

Krug & Wick, 2011

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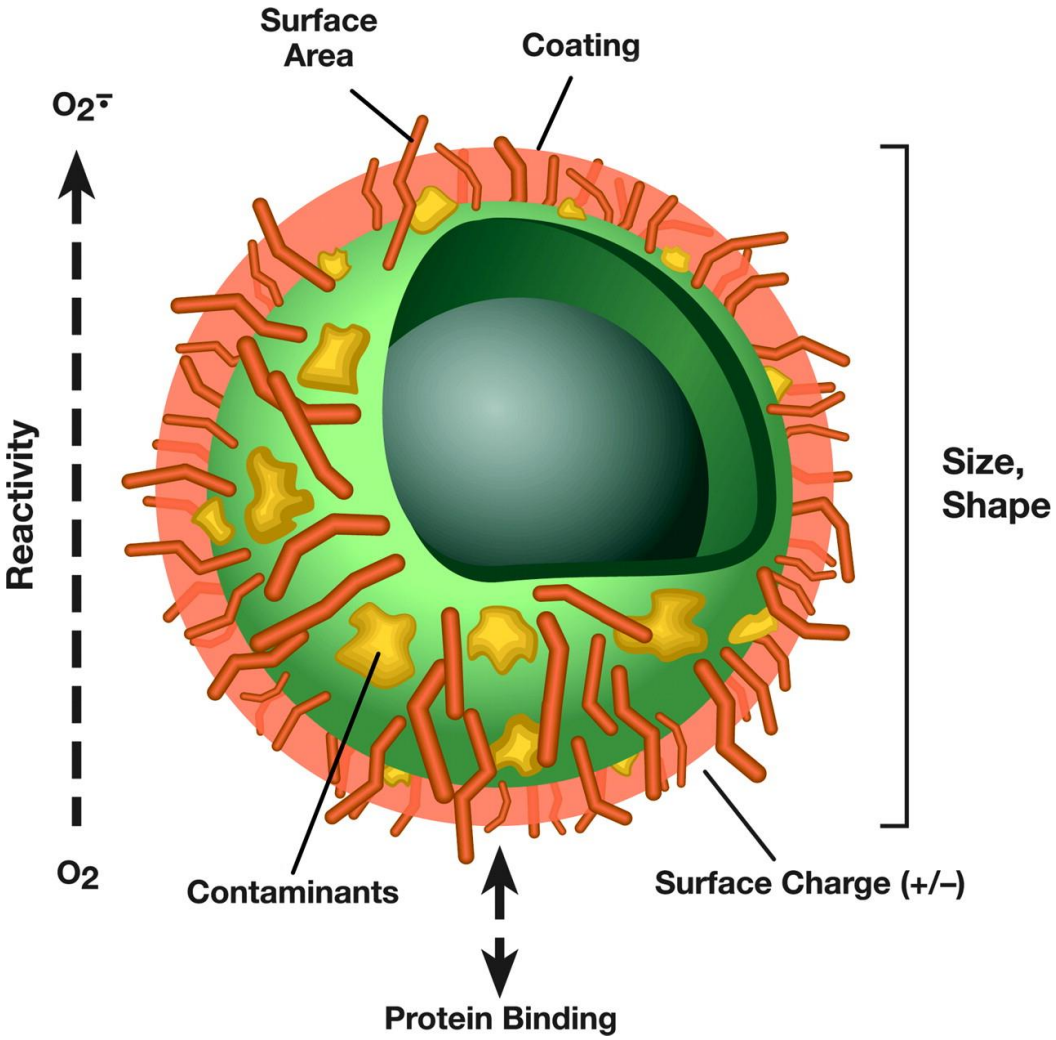
Novelty of Nanoscale Materials means:

- Need for standard terminology
- New analytical methods are needed
  - How to measure and report
  - How to distinguish nanoscale from bulk materials
  - How to measure nanoparticles in matrices
- Need to reassess dose response relationships
- New measurement units may be needed
  - Mass may not be the best indicator of exposure
  - Exposure limits may need to be on the basis of
    - number of particles, or
    - address specific material characteristics

Physicochemical properties of nanoparticles that may influence biocompatibility.



Stern S T , McNeil S E Toxicol. Sci. 2007;101:4-21

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TOXICOLOGICAL SCIENCES

PHYSICAL AND CHEMICAL PROPERTIES

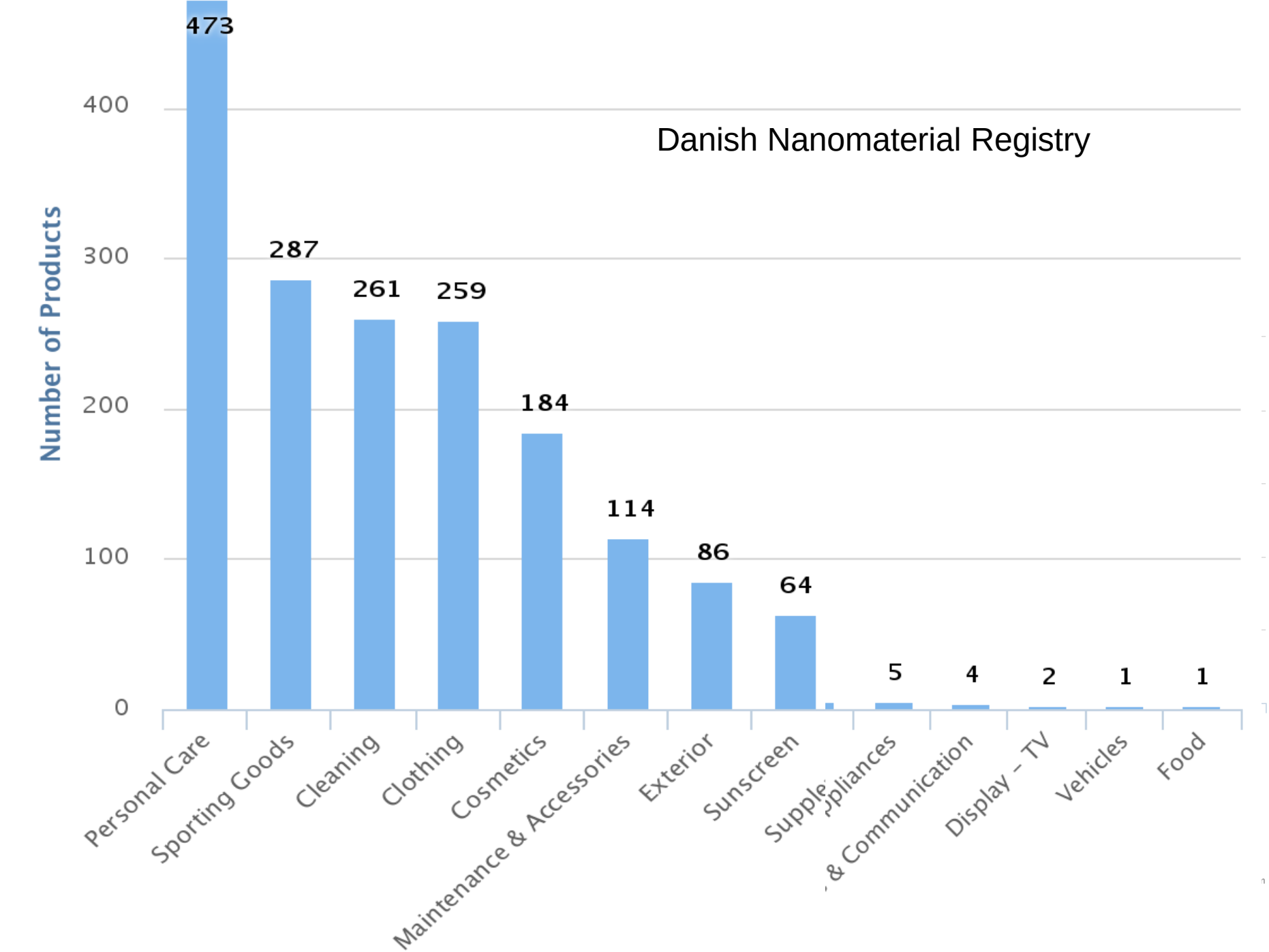
- Higher surface area can mean larger number of active sites
  - Good for modification, but can change behavior
- Surface properties also affect biol./env. behavior?
- Lists – Some guidance exists (*e.g.* ISO 12014:2012)
  - Not universally used
  - Challenges in using measurement techniques
- Measurements in matrices and composites
  - Properties can change in biological and environmental media
  - Nano "release" methods

Recommended physico/chemical characterization parameters for testing toxicity of nanomaterials

CHARACTERISTIC	ILSI 2005	ISO 2010	OECD 2010	Card et al. 2010	Minchar 2009
Agglomeration state/ aggregation	X	X	X	X	X
Particle Size/Distribution	X	X	X	X	X
Composition		X	X	X	X
Shape	X	X		X	X
Solubility/dispersibility		X			X
Specific surface area	X	X	X	X	X
Surface chemistry	X	X	X	X	X
Surface charge	X	X	X	X	X
Porosity	X		X		
Crystal structure	X		X	X	X
Dustiness			X		
Electron Microscopy			X		
Photocatalytic activity			X		
Kow			X		
Redox potential			X		
Radical formation potential			X		
Purity				X	X
Stability					X

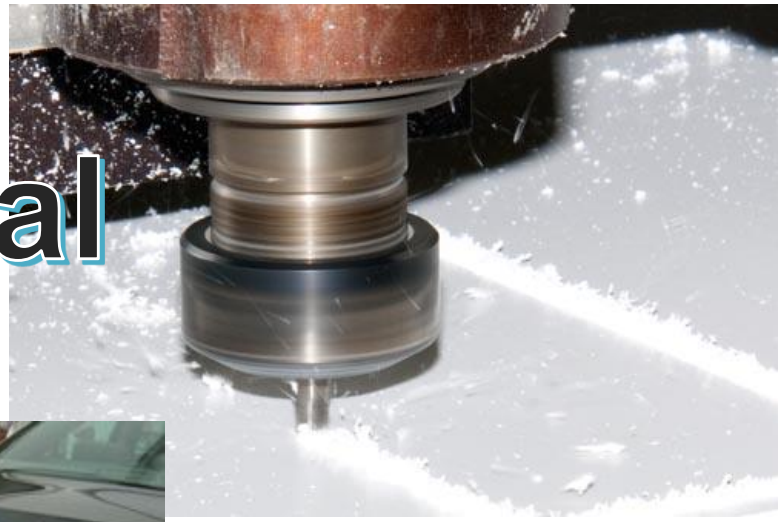
Possible implications of size/surface area changes

- Cross barriers (gut, lung, skin?) more easily than large particles
  - But generally no more than molecules….
  - Except where there is active transport?
- Greater reactivity
  - if the surface is reactive in a particle, then more surface means more reaction
- New reactivity
  - Materials may become catalytic
  - New properties (generally lower than 30nm)
- Increased bioavailablity
  - Different dissolution kinetics for soluble materials


Danish Nanomaterial Registry
Number of Products
0
100
200
300
400
473
287
261
259
184
114
86
64
5
4
2
1
1
Personal Care
Sporting Goods
Cleaning
Clothing
Cosmetics
Maintenance & Accessories
Exterior
Sunscreen
Supple
ppliances
& Communication
Display – TV
Vehicles
Food

EXPOSURE POTENTIAL

Occupational

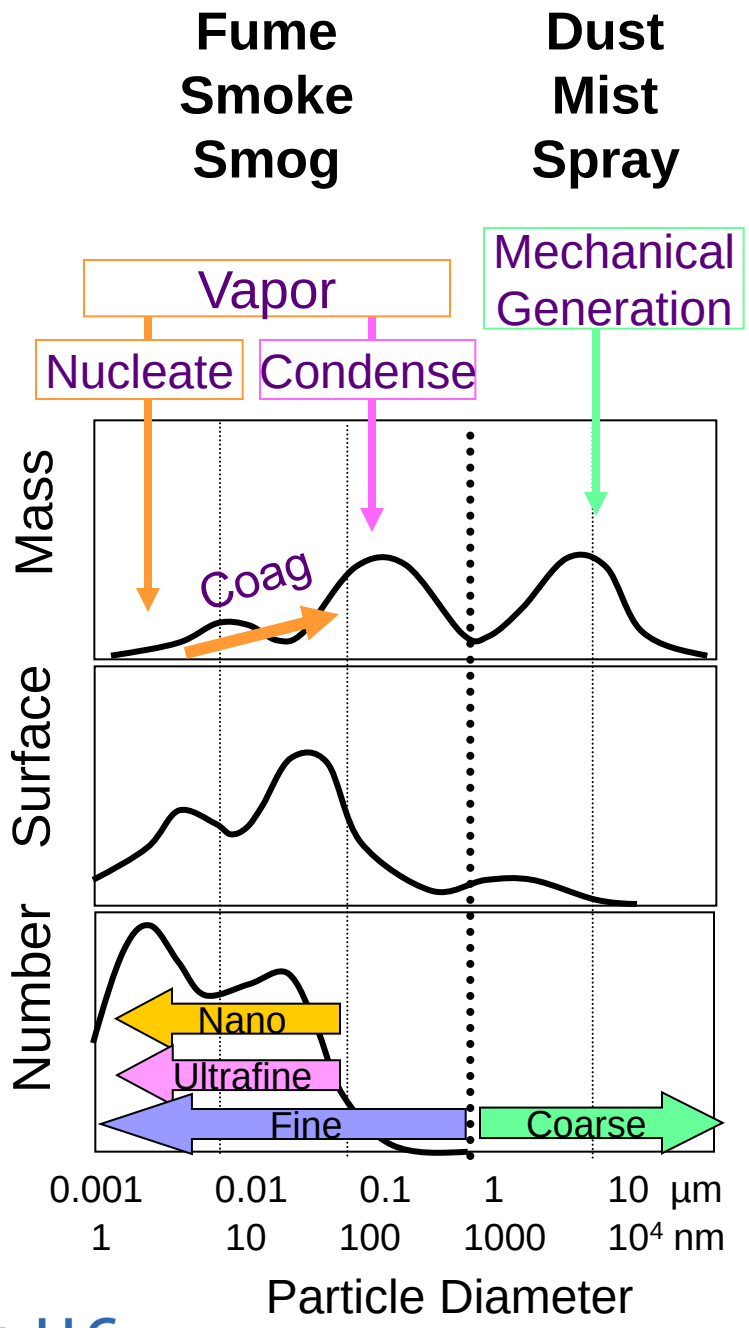


Consumer

Environmental

Generation / Dispersal

- Hot processes
  - Vapor → particle
  - Dp < 1 µm
  - Welding, combusting
- Mechanical processes
  - Dp > 1 µm
  - Grinding, sanding



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Potential Consumer Exposure Pathways

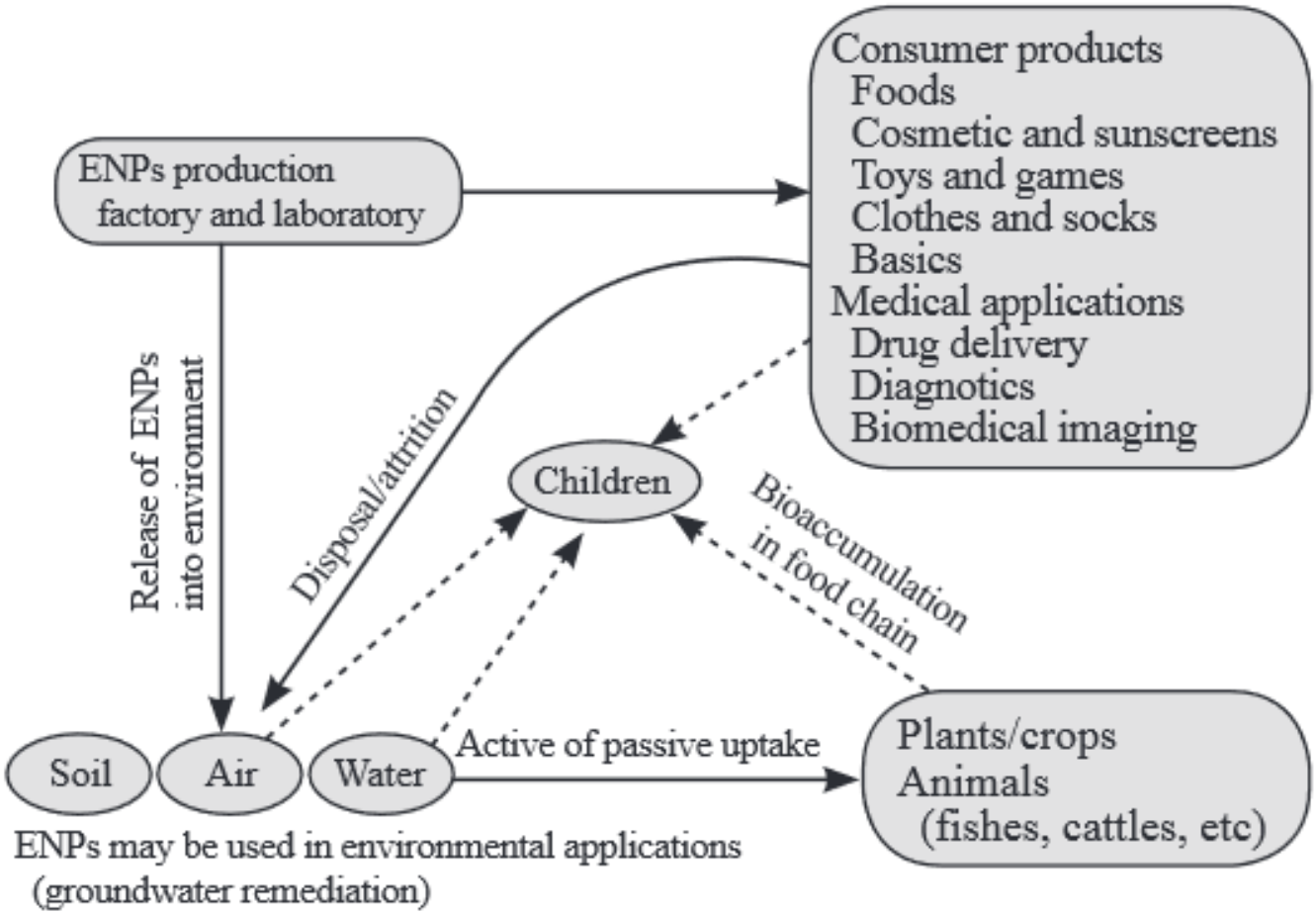


Fig. 2. Potential release, exposure, and uptake of ENPs in children. ENPs: engineered nanoparticles.

Tang, et. al. 2015

Three Principles of Nanotoxicology

- Transport Principle
  - Alternative pathways allow smaller particles to be taken up
- Surface Principle
  - Greater surface reactivity and other surface interactions can increase binding and reactivity with cells
- Material Principle
  - Chemistry of the particle of course affects toxicity

Krug, H. F., & Wick, P. (2011). Nanotoxicology: an interdisciplinary challenge. Angewandte Chemie International Edition, 50(6), 1260-1278.

Hypothesized Uptake Mechanisms for ENPs

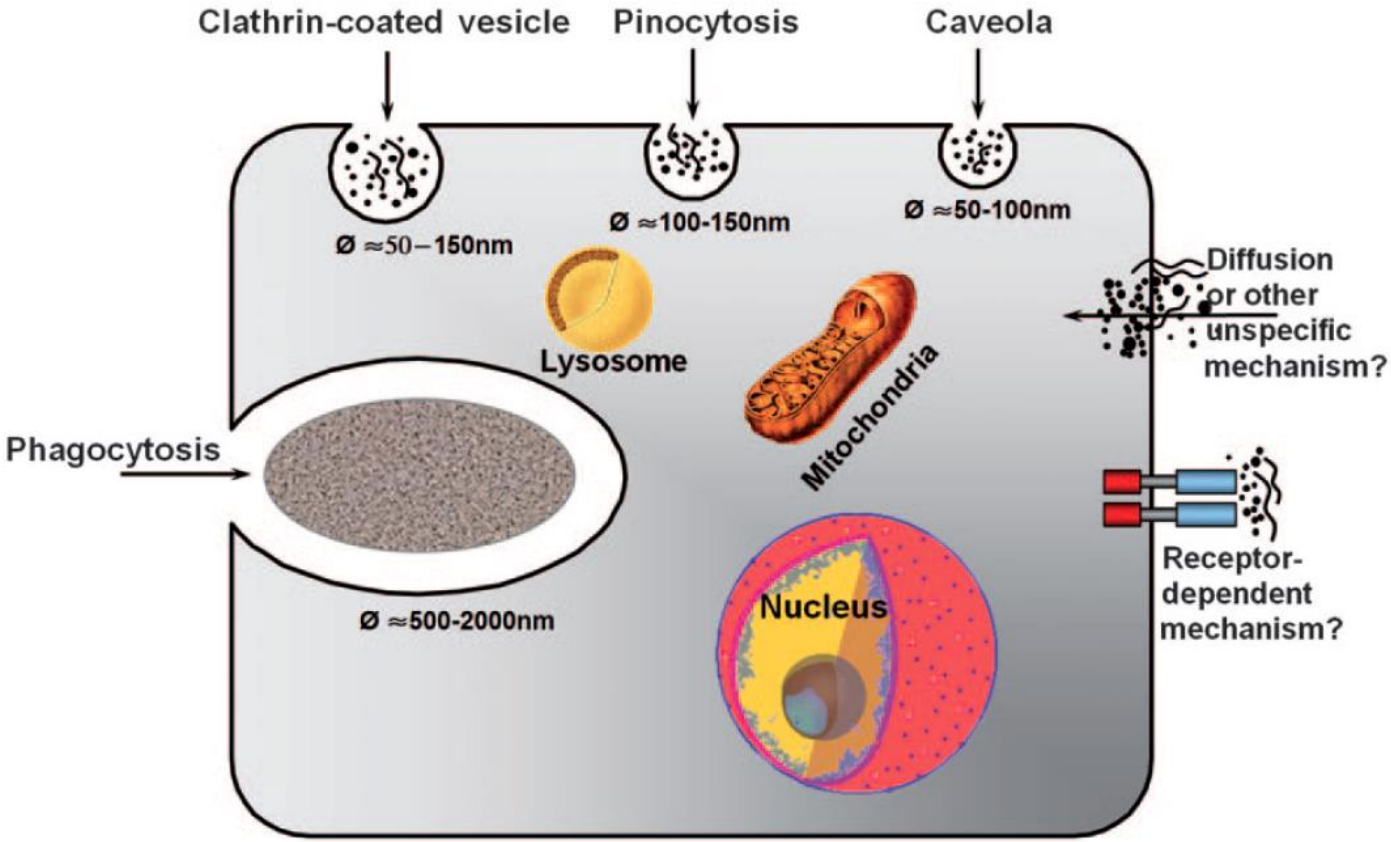
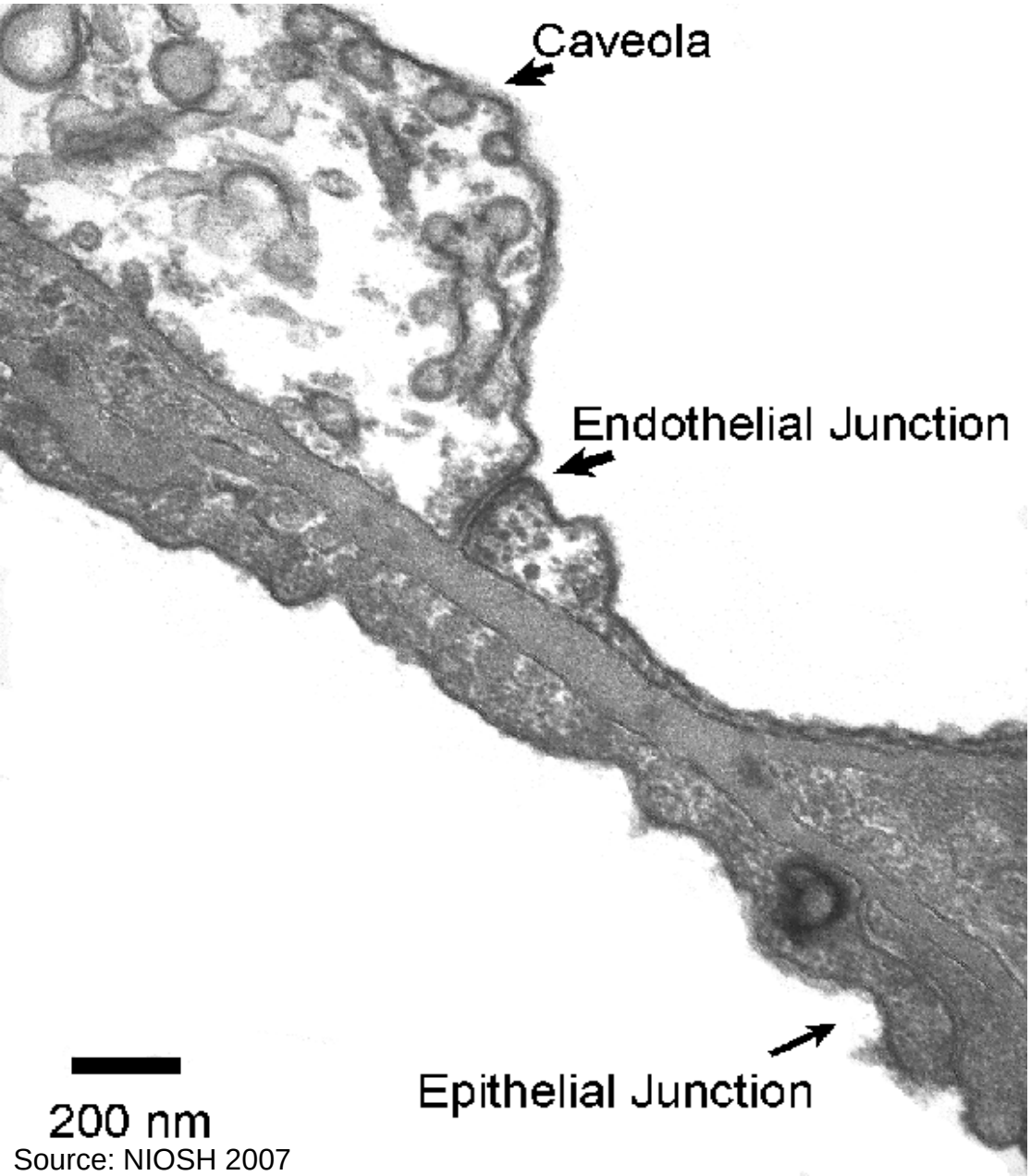


Figure 9. Proposed cellular uptake mechanisms for nanoobjects. In contrast to large particles (>500 nm), which will be exclusively taken up by phagocytosis, nanoobjects may use different translocation routes into the cells. (Modified and reproduced from Ref. [133].)

Krug, H. F., & Wick, P. (2011). Nanotoxicology: an interdisciplinary challenge. Angewandte Chemie International Edition, 50(6), 1260-1278.

Sizes of Nanoscale Materials vs. Alveolar Junctions



Atom 0.1-0.3 nm

Colloidal gold 10 nm

Quantum Dot 30 nm

Large Molecule 70-100 nm

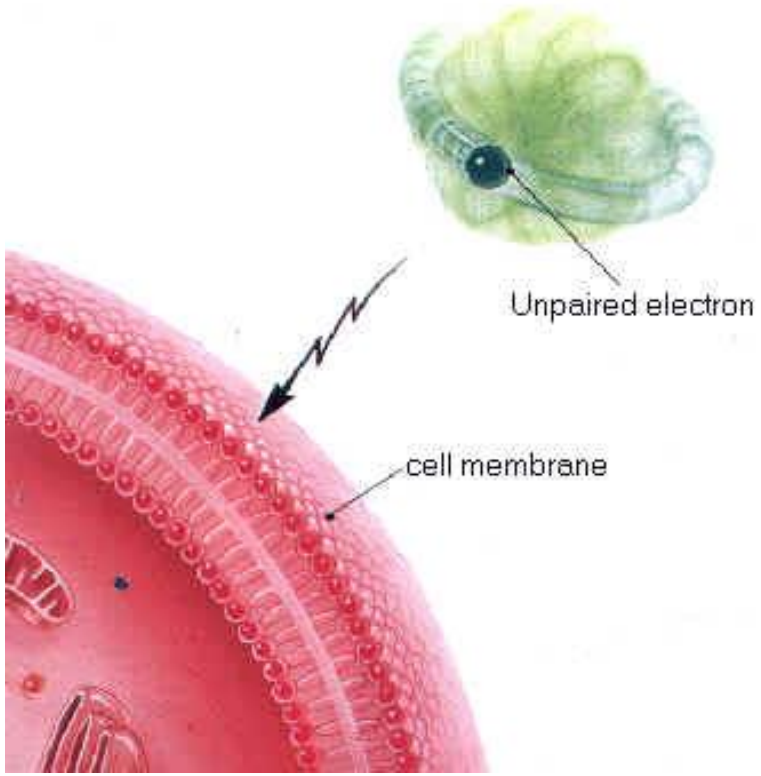
Nanotube 1 nm diameter

Nanorope 15 nm diameter

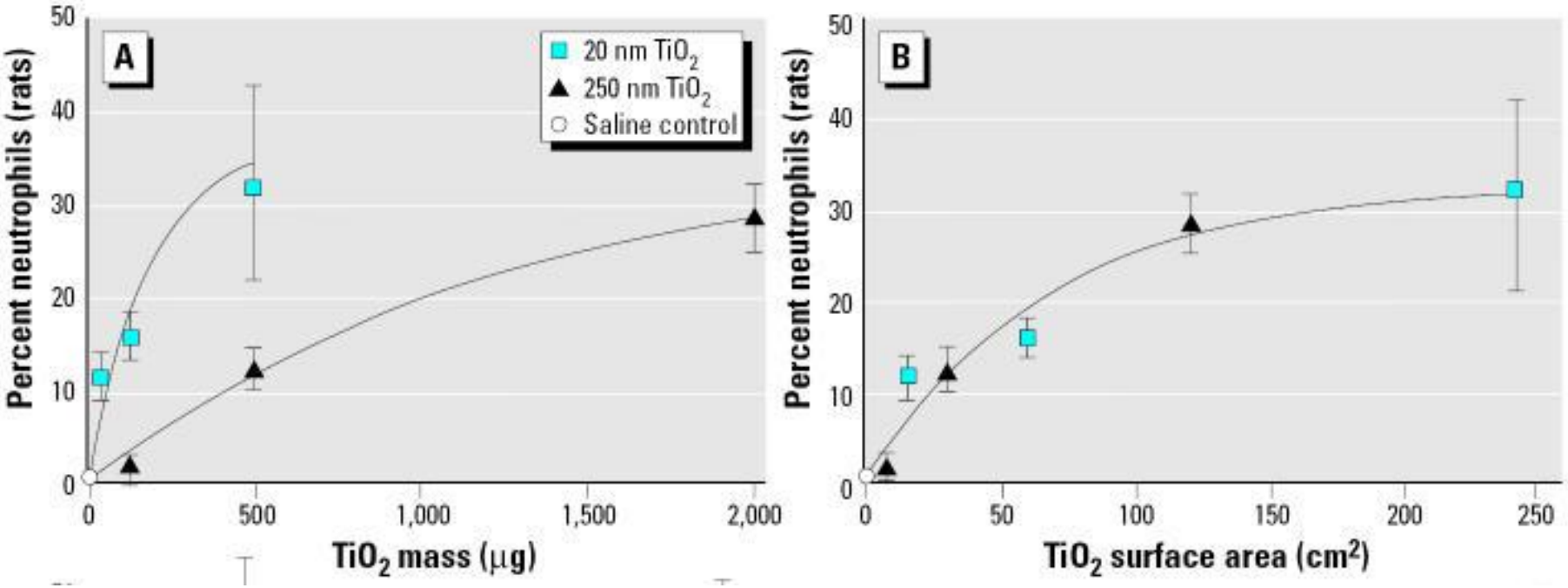
Source: NIOSH 2007

Some Nanoparticles cause Oxidative Stress

- *In vitro* study results indicate that interactions between some ENM and cells produce reactive oxygen species (ROS)
- ROS include
  - Superoxide - $O^{2\bullet-}$
  - Hydroxyl radical - OH^{-}
- Oxidative damage due to interactions between free radicals and cell membranes
  - Can lead to inflammation and diseases



SURFACE AREA CAN BE A BETTER DOSE METRIC FOR SOME NANOMATERIALS



If the 20nm particle aggregated during handling in an assay, would ED50 dose by mass differ? In this case surface area may be a better QA control of effective dose where particle size can vary across dose administrations.

Oberdörster *et* al., 2005. Environ Health Perspect 113(7):823-839.

Potential Lung Transport Pathways

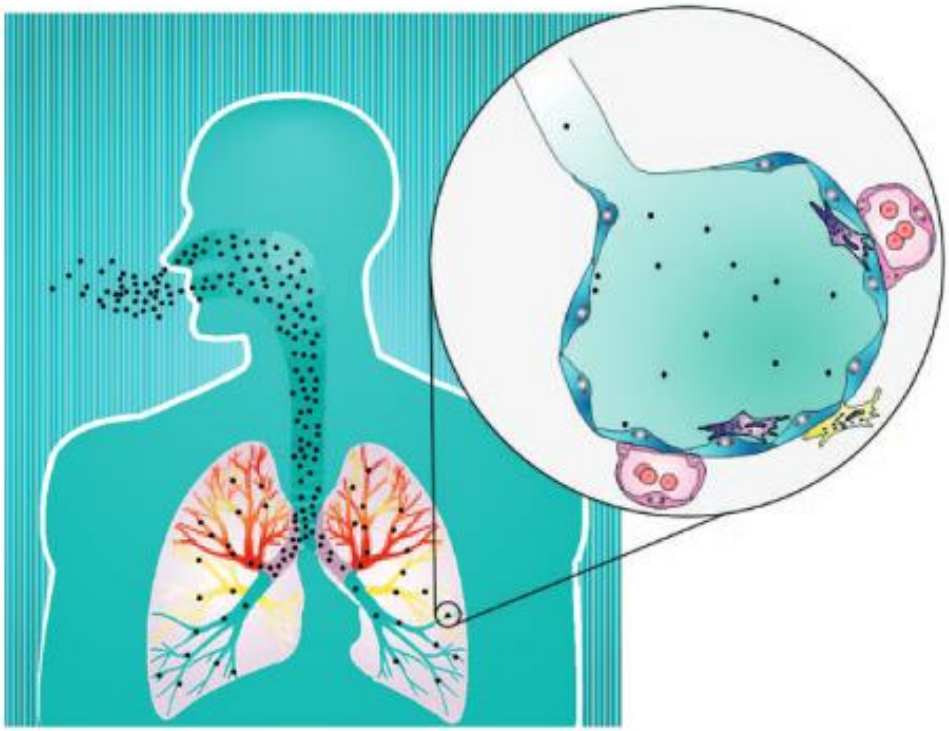


Figure 5. Possible transport pathway for nanoparticles in the lung. Inhaled particles that are smaller than 2.5 μm ($PM_{2.5}$) have access to the alveolar structures of the deep lung and may, in high doses, induce inflammation. A very small portion of the nanoparticles can cross the air–blood barrier and will be distributed via the bloodstream (red). Within the alveoli, most of the particles will be phagocytized by macrophages (purple) or dendritic cells (yellow) or may also be taken up by epithelial cells (blue).

Krug & Wick, 2011

Carbon Nanotube Pathways and Ni^{2+} Dependent Toxicity

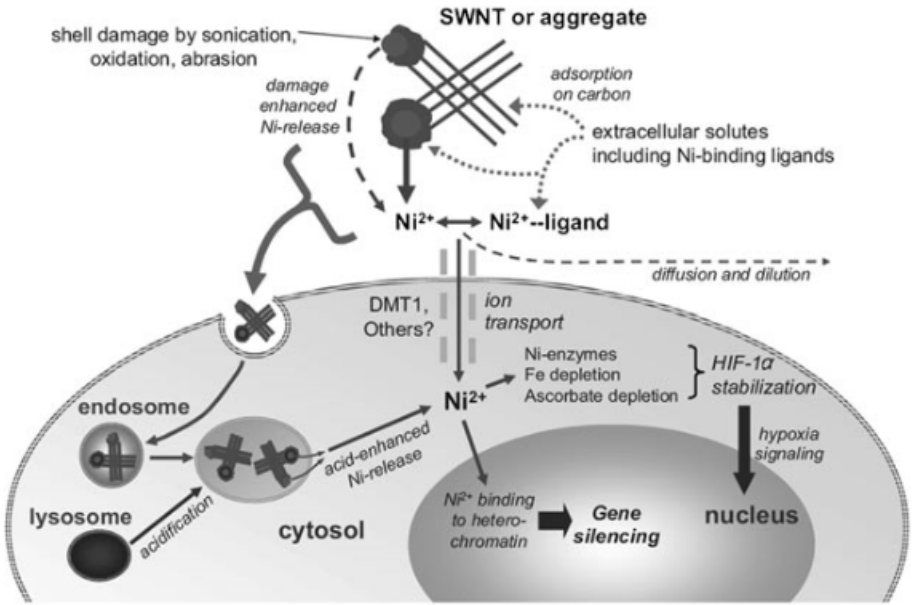


Figure 3. CNTs can act as cell membranes penetrating shuttles, delivering toxic nanoparticles to cells. Transport pathways and molecular toxicity mechanisms associated with cellular exposure to particulate nickel related to issues specific to carbon nanotubes. Intracellular nickel uptake can occur by ion transport or CNT endocytosis/phagocytosis followed by acid-enhanced Ni^{2+} release in lysosomes. The biologically active species is reported to be Ni^{2+}, which disrupts the hydroxylation of transcription factor HIF1-α or induces gene silencing by binding to heterochromatin. The most significant unknown in this Scheme is the nickel release behavior of CNTs (bioavailability), which should be characterized under both extra- and intracellular conditions. The bioavailability of CNT nickel might also depend on Ni-binding ligands, which affect both mobilization and cellular uptake. Reprinted with permission from [23].

Hurt & Kane, 2007

GI Tract Component Conditions Affecting Ingested ENPs

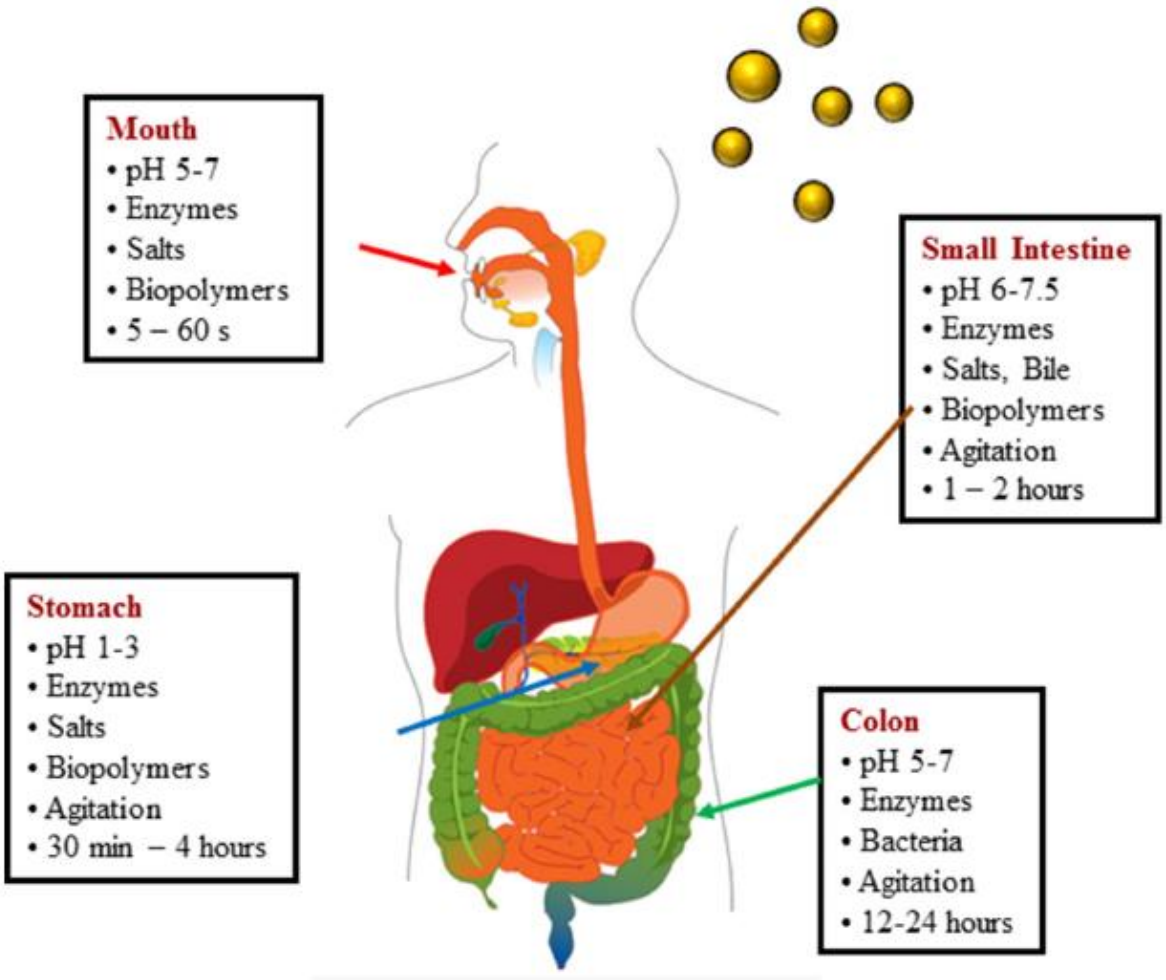


Fig. 3. Schematic diagram of the physicochemical and physiological conditions in different regions of the human gastrointestinal tract. The diagram of the human body was taken from http://en.wikipedia.org/wiki/Digestive_tract (Copyright free).

McClements et. al. 2016

Potential Changes to ENP Properties in the Gut

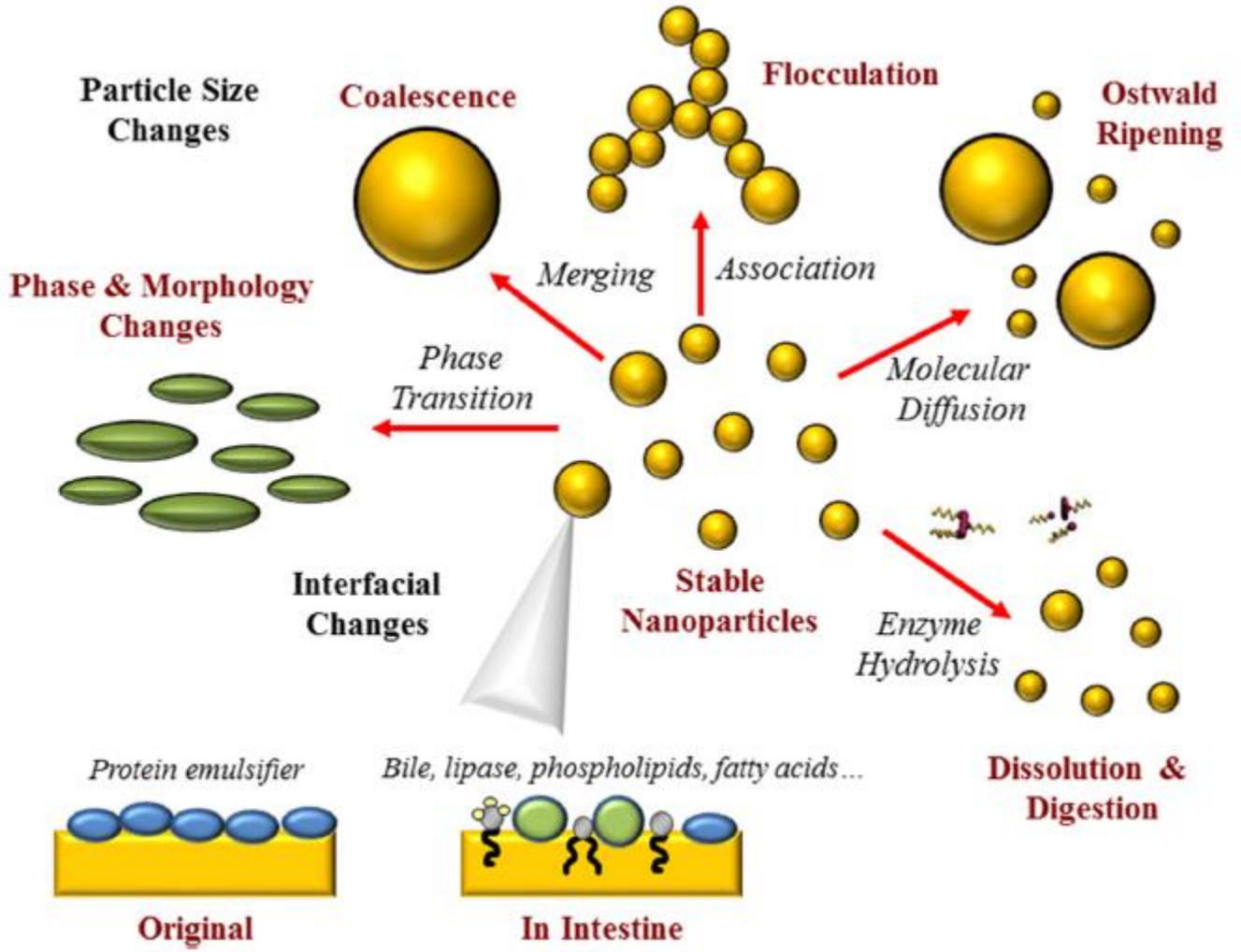


Fig. 4. The properties of nanoparticles may occur in a number of different ways as they pass through the GIT. Some potential changes in particle dimensions and interfacial properties are illustrated here.

McClements et. al. 2016

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Absorption Distribution Metabolism and Excretion (ADME) Knowledge Gaps for Nanomaterials

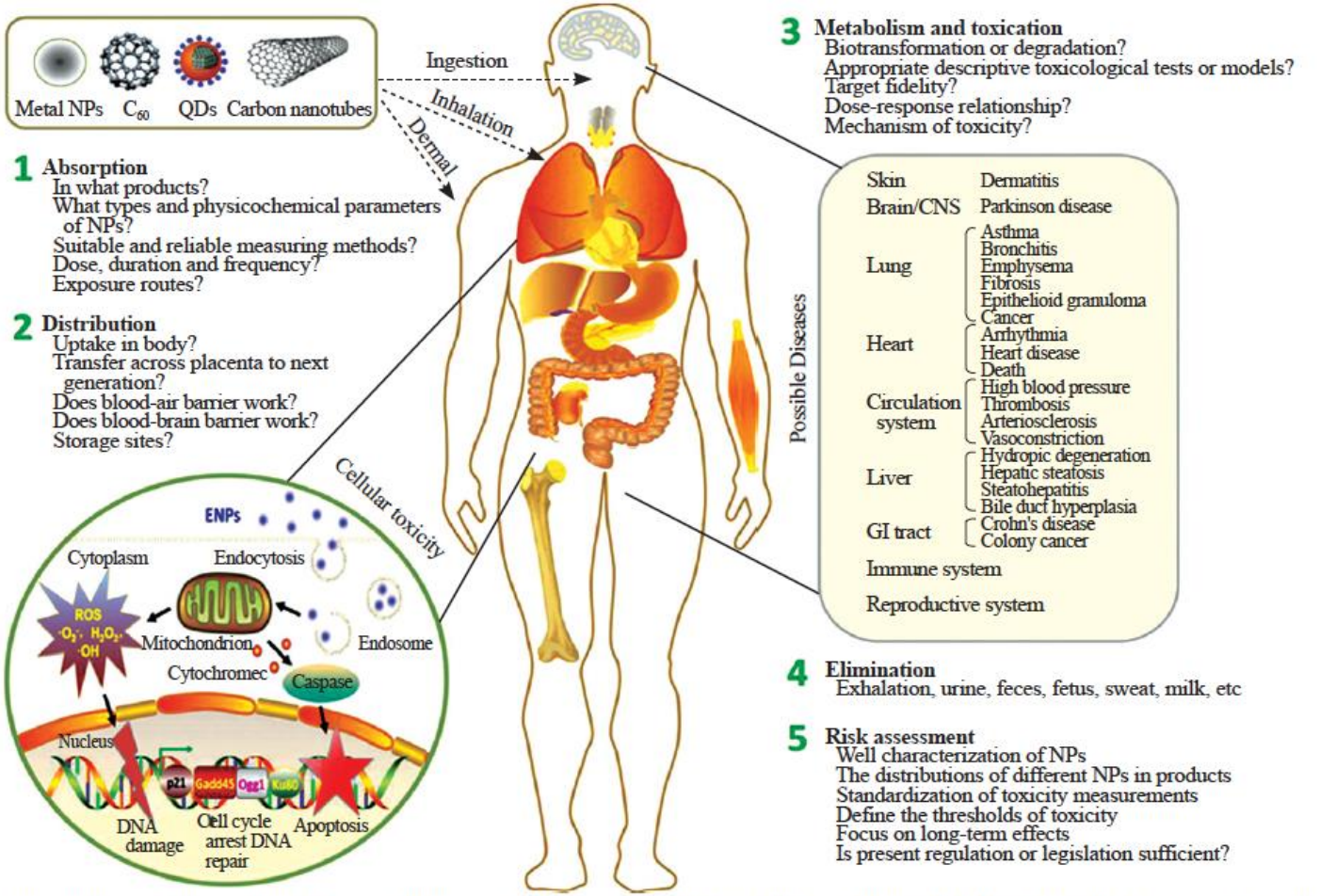

Fig. 3. Knowledge gaps intoxicokinetic, toxicodynamic, and risk assessment data on ENPs in infants and children. Possible diseases were mainly based on a previous review.[5] NPs: nanoparticles; QDs: quantum dots; ENPs: engineered nanoparticles; ROS: reactive oxygen species; CNS: central nervous system; GI: gastrointestinal; O_2: oxygen; H_2O_2: hydrogen peroxide; OH: hydroxyl; p21: cyclin-dependent kinase inhibitor 1; Gadd45: growth arrest and DNA-damage-inducible protein 45; Ogg1: 8-oxoguanine DNA glycosylase; Ku80: X-ray repair cross-complementing 5.

Source: Tang et al. 2015 Health implications of engineered nanoparticles in infants and children, World J Pediatr 11(3):197-206

In a review of more than 10,000 publications from 2000-2014; main findings of *Nanosafety Research – Are We on the Right Track?*

Despite number of studies conducted, no clear statement on safety of nanomaterials

– many studies are self contradictory and/or have design flaws
– many studies did not characterize materials limiting their use
– need standards for physicochemical characterization in studies
– studies often don't take dissolution of NMs in body fluids into account; no indication of nano-specific toxicity

Emerging trends:

– ENMs are taken up in the lung and GI tract but only small fraction reaches circulation and most are cleared before uptake. Reports of systemic effects are rare.
– Instillation and inhalation experiments commonly report transient inflammatory effects similar to fine dusts with exceptions (high aspect ratio materials); instillation experiments often meet "overload conditions".

Krug, *Angew. Chem. Int. Ed*. 2014, 53, 12304 – 12319

Good Nanotox Studies Should Report

- Applied quantities (concentration/dose), to be given in more than one unit and expressed as: mg/mL , mg/cm2 , N (particle)/cell , pg/cell.
- Doses administered during animal experiments should be clearly marked as “overload” or “non-overload” doses. Overload doses should be largely avoided as they impede unambiguous statements.
- At least two different tests should be made for each biological end point to exclude cross-reactions.
- As unspecific cell reactions (for example, apoptosis) can cause DNA damage, cytotoxic concentrations should be avoided in genotoxicity studies. Any such study should contain data on the dose–effect relationship of the acute toxic effects.
- Interference of the nanomaterials with the test system should be taken into account in any case and be excluded if possible.
- Paths of uptake and an appropriate selection of experimental organisms should also be considered when performing ecotoxicological studies.

Krug and Wick (2011)

ENVIRONMENTAL ISSUES

- Transformations & kinetics
  - In complex natural environments, ENMs bind readily to proteins and other organic matter, continually changing physical/chemical properties & behavior
  - Dynamics of release of ions can be affected
- Environmental fate
  - Detection in environmental media very difficult
- Toxicity
  - Need to understand fate, actual exposure dose and form, and mechanism of effect
  - Current standardized tests for toxicity testing can be affected by nanomaterials
- End of life
  - Gaps in knowledge
- Methods for composite materials
  - Migration/leaching from the products

State of Nano-Eco Tox/Exposure/Risk

Table 1. Moving nanoparticle environment, health, and safety research forward: Creating a new framework to assess the environmental impact of manufactured nanomaterials

	State of science	Gaps	Framework
Detecting nanomaterials	• In pristine conditions	• In complex media • At realistic concentrations • For aged or weathered materials • Relative to background materials	• Develop colloid science techniques for environmental matrices • Gather input from toxicologists on appropriate metrics
Predicting fate	• Behavior of pristine, unaltered nanomaterials in laboratory settings	• The nature of released particles • Information on nanoparticle being altered and aging in the environment • Product-specific particle processes and time scales	• Assess exposure for product and/or altered nanoparticle-specific categories of nanomaterials
Assessing hazard	• Endpoints and relevant species	• Sufficiently fast and targeted analytical methodology to meet data needs during testing • Appropriate controls • Addressing time-dependent exposure • Dispersion methods • Scale (volume) problems	• Apply newly developed technology for exposure monitoring and control • Account for time-varying exposure • Prioritize toxicology tests most likely to identify risks • Develop minimum toxicology recommendations
Developing risk assessments	• Existing framework available and applicable • Limited scientific information • First global approaches for screening assessment	• Exposure uncertainty because of uncertain fate processes • Uncertain effects thresholds • Uncertainty of risk characterization metrics • Tools for location-specific assessment	• Examine product vs nanoparticle vs aged nanoparticle • Address physical form and spatial variability • Investigate interactions with toxic chemicals • Consider nanoparticle-type specific metrics

Klaine, et. al. 2012

Advancing Risk Analysis for Nanomaterials

A Workshop to Explore How a Multiple Models Approach Can Advance Risk Analysis of Nanoscale Materials

September 15-16, 2014
Milken Institute School for Public Health
George Washington University, Washington, DC

American Chemical Society
Center for the Environmental Implications of NanoTechnology
George Washington University Milken Institute for Public Health
Society for Toxicology Nanotoxicology Specialty Section
Society for Toxicology and Chemistry Nanotechnology Advisory Group
Sustainable Nanotechnology Organization
University of California Center for Environmental Implications of Nanotechnology

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SRA Nano Risk Workshop Resources

Risk Analysis
AN INTERNATIONAL JOURNAL
An Official Publication of the Society for Risk Analysis

Special Issue: Alternative Testing Strategies for Risk Assessment of Nanomaterials
August 2016 36 (8): 1511–1681

Introduction to Special Series (pages 1518–1519) Jo Anne Shatkin

Advancing Risk Analysis for Nanoscale Materials: Report from an International Workshop on the Role of Alternative Testing Strategies for Advancement (pages 1520–1537) J. A. Shatkin, Kimberly J. Ong, Christian Beaudrie, Amy J. Clippinger, Christine Ogilvie Hendren, Lynne T. Haber, Myriam Hill, Patricia Holden, Alan J. Kennedy, Baram Kim, Margaret MacDonell, Christina M. Powers, Monita Sharma, Lorraine Sheremeta, Vicki Stone, Yasir Sultan, Audrey Turley and Ronald H. White

Approaches to Develop Alternative Testing Strategies to Inform Human Health Risk Assessment of Nanomaterials (pages 1538–1550) Vicki Stone, Helinor J. Johnston, Dominique Balharry, Jeremy M. Gernand and Mary Gulumian

Framework to Evaluate Exposure Relevance and Data Needs for Risk Assessment of Nanomaterials using *in Vitro* Testing Strategies (pages 1551–1563) Monita Sharma, Jo Anne Shatkin, Carolyn Cairns, Richard Canady and Amy J. Clippinger

Alternative Testing Strategies for Nanomaterials: State of the Science and Considerations for Risk Analysis (pages 1564–1580) J. A. Shatkin and K. J. Ong

Case Study of nanoscale Titanium Dioxide (n-TiO_2)

Analysis of 1820 results from 96 publications of *in vitro* and *in vivo* studies*

Types of TiO_2

Commercial: 133 (64%)
Made in-lab: 57 (27%)
Unknown (not reported or recorded): 19 (9%)

Are studies using environmentally relevant concentrations?

Aquatic:
PEC: 0.016 mg/L (Mueller and Nowack 2008)
3% of studies (1 of 35) use a concentration < 0.016 mg/L

Inhalation:
PEC: 0.042 µg/L (Mueller and Nowack 2008)
0% of studies (0 of 58) use a concentration < 0.042 µg/L.

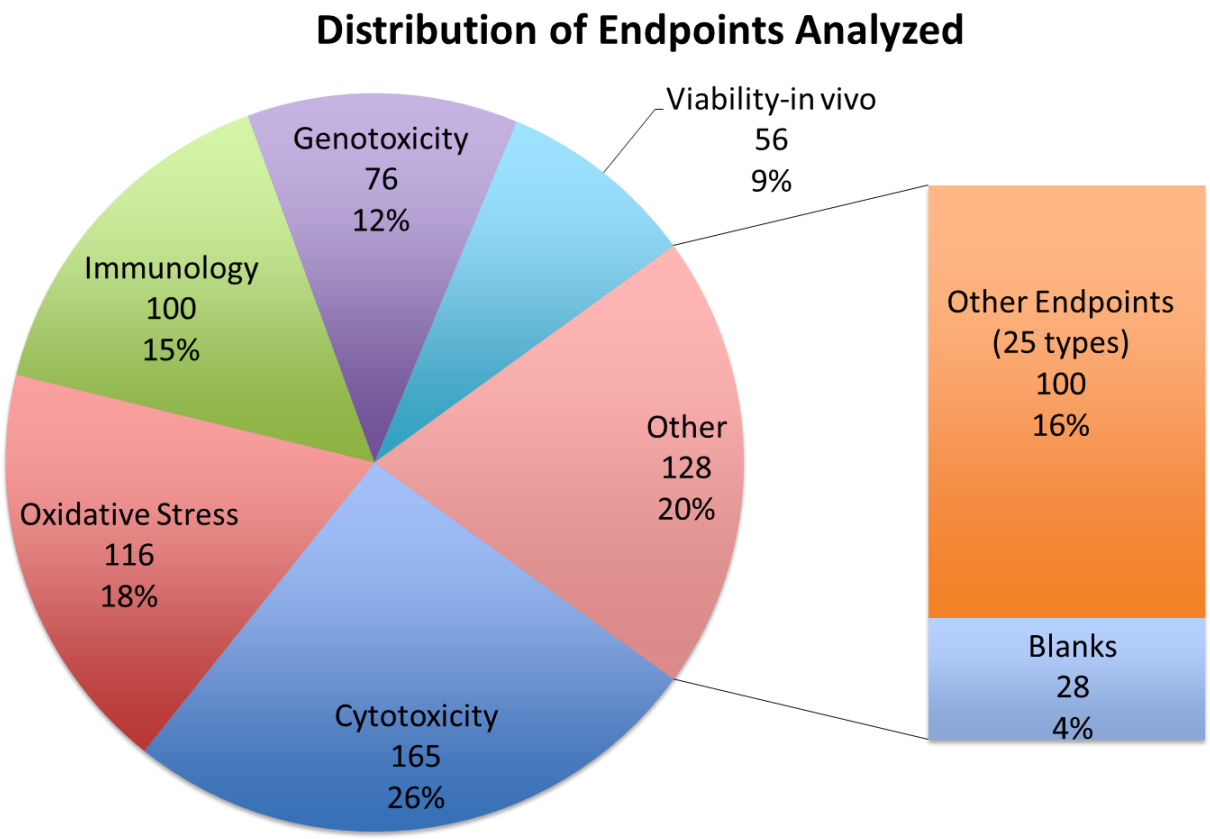
Ingestion:
Max 3 µg/kg-bw/day (roughly 36% of the particles may be nano) (Weir et al. 2012); roughly 0.1 mg TiO_2 person/day nanoscale TiO_2
0% of studies (0 of 8) use < 3µg/kg/day. The studies ranged from 5-5000 mg/kg/day

Endpoints analysed	
Top 5 types of studies	
Cytotoxicity:	165 (25.7%)
Oxidative stress:	116 (18.1%)
Immunology:	100 (15.6%)
Genotoxicity:	76 (11.8%)
Viability (in vivo):	56 (8.7%)

**** INCLUDING WPMN DRAFT DOSSIER***

Shatkin JA, Ong KJ. Alternative Testing Strategies for Nanomaterials: State of the Science and Considerations for Risk Analysis. *Risk Anal.* 2016 Aug;36(8):1564-80.

Case Study Analysis with nano TiO2



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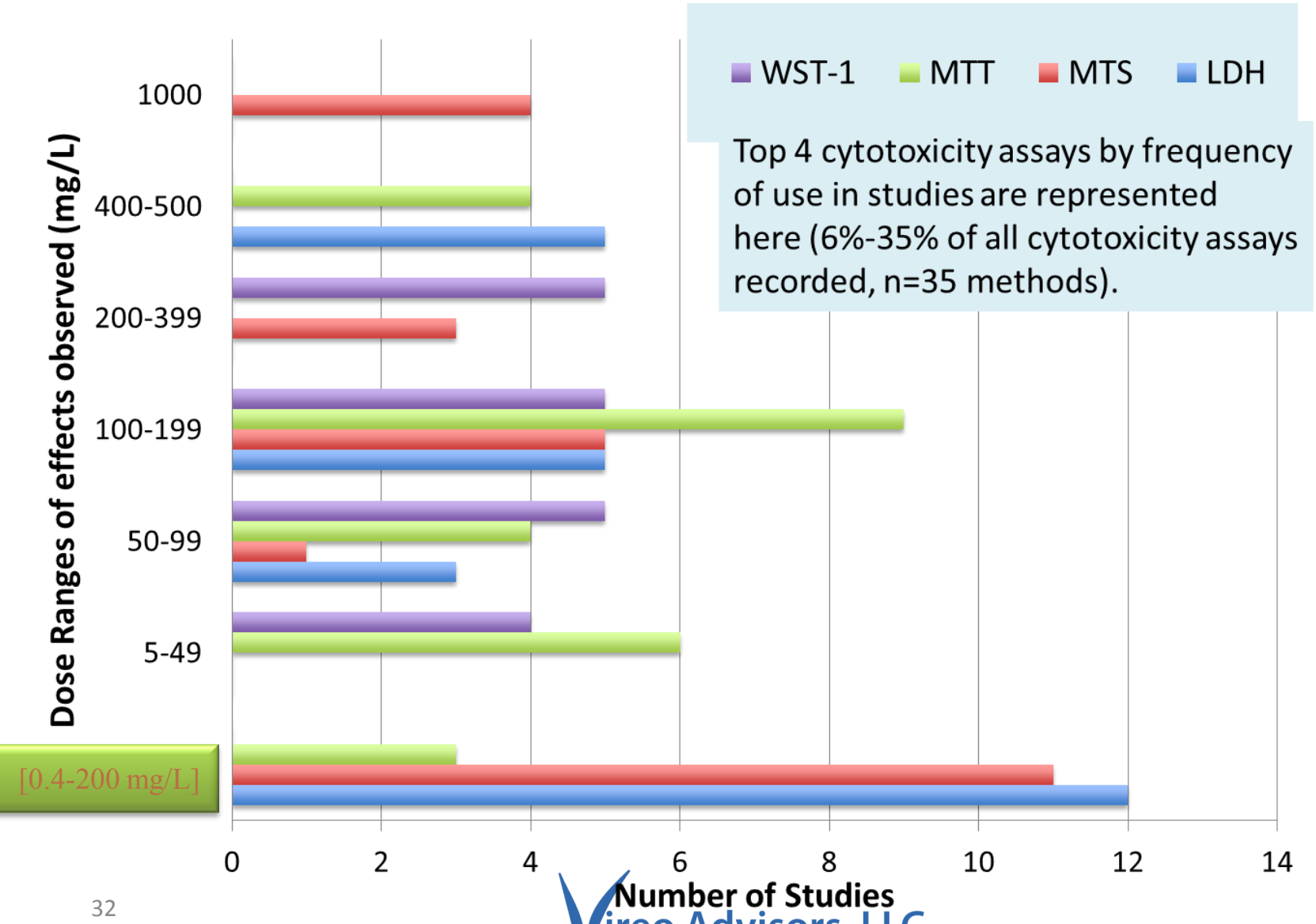
Cytotoxicity Assays and Dose Effect Observed



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OECD Working Party Manufactured Nanomaterials (WPMN) Project Proposal ENV/CHEM/NANO(2016)2222

Advancing Adverse Outcome Pathway (AOP) Development for Nanomaterial Risk Assessment and Categorization

LEAD COUNTRY -CANADA

Environment and Climate Change Canada

Health Canada

Alberta Innovates Technology Futures

COLLABORATORS

Vireo Advisors (USA)

The Federal Institute for Risk Assessment (BfR)

Dutch Technical University (Netherlands)

Vireo Advisors, LLC

OVERALL PROJECT APPROACH

APPROACH

Building on outcomes of *ATS Pilot Project**, **develop a methodology** and **apply it in a case study on a key event in the inflammation AOP pathway** to **highlight how AOP frameworks can be used in a regulatory context to inform future categorization and risk assessments of MNs**. Case study focuses on a commonly activated pathway following MN exposure: inflammation.

** OECD. 2016.* **ENV/JM/WRPR(2016)63.** *ADVANCING THE PRACTICE OF RISK ASSESSMENT WITH ALTERNATIVE TESTING STRATEGIES: STATE OF THE SCIENCE FOR READ ACROSSs AND RISK ASSESSMENT GUIDANCE (not declassified)*

Framing the Issues: Hazard Characterization for Nanomaterials

- How to define nanomaterials
  - Distinguish engineered from other nanoparticles?
  - Are agglomerated or aggregated particles nano?
  - Is a composite material containing nanoparticles "nano"?
- Do we characterize the particle, or the product?
  - Lack of standardization
- What are the appropriate measurement units?
- How to characterize variability, uncertainty?

Framing the Issues: Exposure Characterization for Nanomaterials

- Need new ways to characterize exposure
  - Mass may not be most useful measure
  - When does size trigger new measures?
  - How does the matrix affect exposure?
- Limitations of available analytical techniques
  - Methods require low detection limits
  - Also need to characterize “background” exposures
- Limited data on transport and fate
- Necessity to improve realism of exposure types and levels

Framing the Issues: Dose Response for Nanomaterials

- Limited data available from well designed studies
  - most is in vitro or inhalation studies to particles
- Reactive oxygen species (ROS) formation is a commonly observed mechanism of toxicity; physical effect on cells
  - Suggest particle effects, beyond chemical behavior
- Study conditions affect results
- Surface coating/particle size/surface charge/ surface area/ contamination and aggregation affect biological and environmental behavior

Health effects and toxicology

- "Unique properties" raise concerns about effects
- Studies generally at high dose levels
- Most studies on raw materials "as produced"
- Active surfaces attract biological molecules
- A few examples:
  - CNT – shape and persistence raise concerns
    - PEL established; risk assessments; IARC listing (MWCNT-7)
  - Nano-silver – antimicrobial; toxicity mainly relates to ion releases

Framing the Issues: Characterizing Risks of Nanomaterials

- Several deliberations conclude that current frameworks adequate and appropriate
  - but require modifications to address particle aspects
- Still much research to be done to quantify risks
- Need to address uncertainty and variability
- Still a limited ability to conduct quantitative assessments
- New metrics and endpoints for risk?

State of the Science for Nanomaterial Safety

- Occupational exposure/risk management strategies exist
- Risks vary across the material/product life cycle
- Number of studies increasing exponentially but low level of standardization – limited characterization of physical and chemical parameters remains an issue
- Measurement techniques lag and are not standardized
- Unclear how to extrapolate findings from one nanomaterial to another – lack predictiveness
- "nanoness" still elusive, yet getting defined

Acknowledge the Vireo Advisors Team

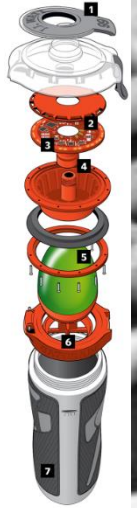
Dr. Jo Anne Shatkin is an expert in nanotechnology safety, environmental and health policy issues, with over 20 years experience leading projects in risk analysis, safety and regulatory policy work including numerous publications. She is founder and president of Vireo Advisors in Boston, Massachusetts.

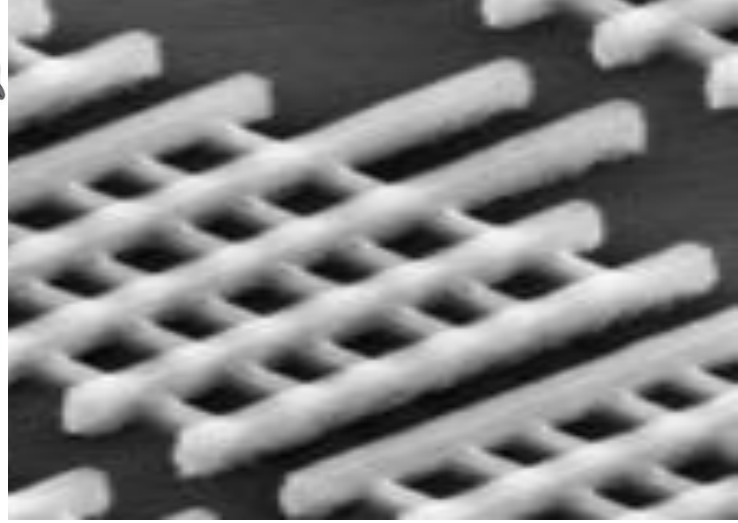
Dr. James D. Ede is a nanotoxicologist experienced in testing strategies for nanomaterials, including molecular, biochemical and cellular techniques to help evaluate the hazard of several high-aspect ratio nanomaterials such as carbon nanotubes and cellulose nanocrystals.

Dr. Kimberly J. Ong is an expert in nanoparticle research and toxicology, particularly in protocol development, *in vivo* aquatic research, and *in vitro* testing. Dr. Ong has experience assessing and modifying protocols specific for nanomaterials' testing to improve reliability for risk and exposure assessment.

Leslie Hockman
Administrative Assistant

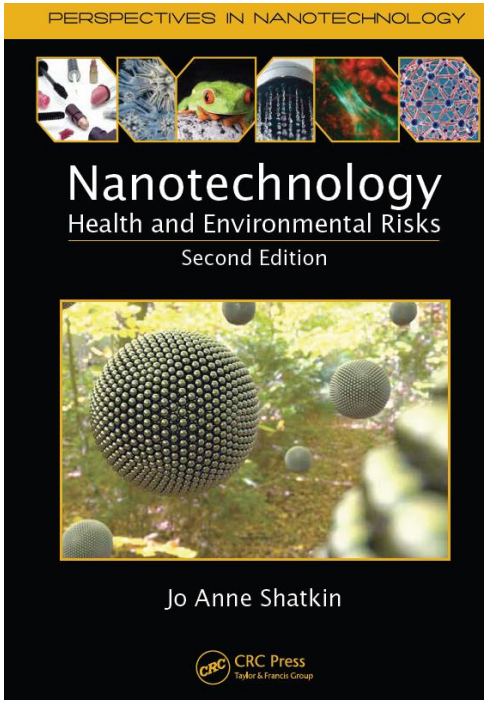






Thank you – let's discuss!

Jo Anne Shatkin, Ph.D.
President
Vireo Advisors, LLC
Boston, MA
jashatkin@VireoAdvisors.com



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