Nanotechnology & Pet Food: What is it? Defining it's importance to you.

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Thank you for the opportunity to speak to you today.

This material was presented by Dr. George A. Burdock at the Pet Food Forum, April, 16, 2013.

If you would like to receive additional information regarding Burdock Group's capabilities in food and feed ingredients, or dietary supplement safety or regulations governing any of the preceding, please contact us at www.burdockgroup.com.

Disclaimer: Concepts and examples discussed in this lecture are for illustrative purposes only and do not constitute regulatory advice. The examples provided herein may not apply to your specific situation. Please contact us for an analysis of your particular circumstance to determine the best pathway for your product and team.

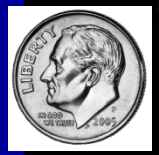
What is Nanotechnology?

- "…the manipulation, precision placement, measurement, modeling or manufacture of sub-100 nanometer scale matter…"
  - *European Commission, 2001*

- "Nanotechnology is the study of phenomena and fine-tuning of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale."
  - *European Commission Nanotechnology Website, 2009*

How big is a Nanometer?

- A meter ≃ 39 inches
- A nanometer is to a meter ----
- As the diameter of a dime
- Is to the diameter of the earth



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Not to scale

How big is a Nanometer?

- Diameter of a hair = 60,000 nanometers
- Diameter of red blood cell = 6,000 nanometers
- Zinc oxide particle for sun block = 20 nm
- Width of strand of DNA = 2-12 nanometers
- Diameter of carbon nanotubule = 1.2 nanometer
- Diameter of carbon nanosphere = 1 nanometer

What's the Big Deal about Nanotech?

[Because] at the nanoscale, the physico-chemical and biological properties of materials differ in fundamental and valuable ways from the properties of individual elements and *molecules of bulk matter*.

National Nanotechnology Initiative

http://www.nano.gov/html/facts/whatIsNano.html

Nanotechnology Fundamental Differences

Physico-Chemical Properties

- Gold
  - Is no longer yellow (red at 3 nm and blue at 1 nm)
  - It becomes catalytic
  - MP drops from 1200 degrees to 200 degrees
- Aluminum becomes combustible
- Copper becomes an insulator

Nanotechnology:
Fundamental Changes Occur

"It's like you can shrink a cat and keep shrinking it, and then at some point, all at once it turns into a dog."

National Geographic, June 2006

Examples of Nanomaterials used as Raw Materials, Intermediates and Final Products

Nanomaterials

Nanoscaled structures in unprocessed form

Such as:

- Carbon nanotubes
- Ceramic nanoparticles
- Dendrimers
- Fullerenes
- Metal nanoparticles
- Nanostructured metals
- Nanowires

GAO Report, May 2010

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Nano-intermediates

Intermediate products with nanoscale features

Such as:

- Catalysts
- Coatings
- Composites
- Displays
- Drug delivery systems
- Energy storage
- Sensors

GAO Report, May 2010

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Nano-enabled products

Finished goods incorporating nanotechnology

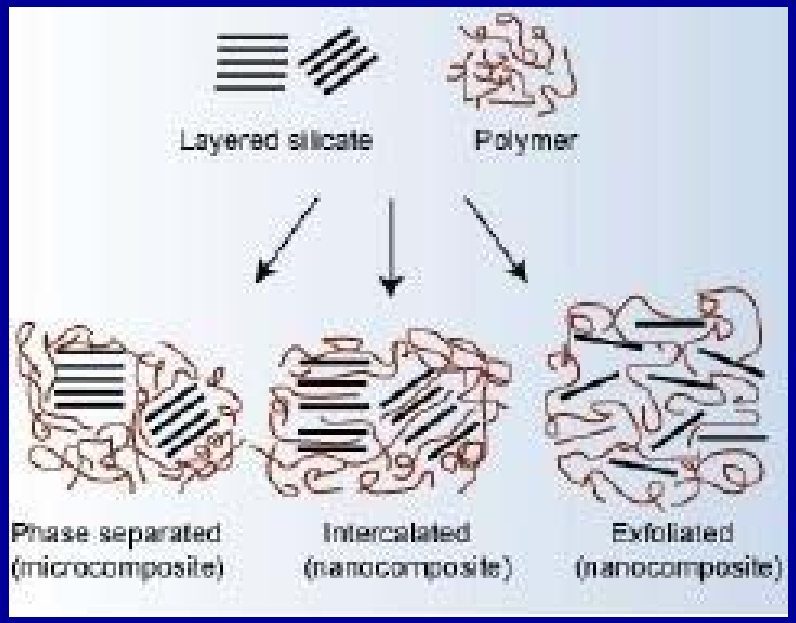
Such as:

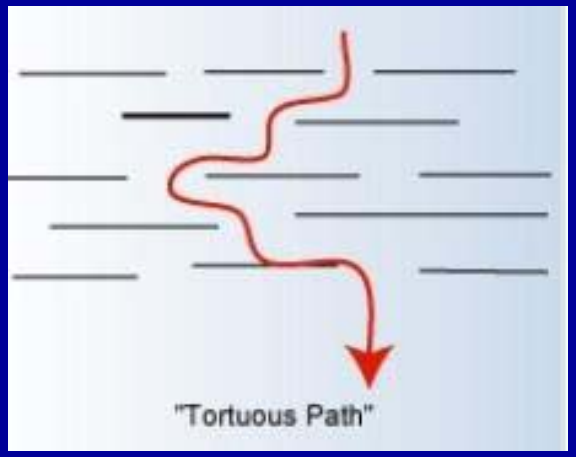
- Automobiles
- Bottles
- Sun block
- Active packaging
- Windows
- Cancer treatment
- Mobile phones

GAO Report, May 2010

Example: Clay Nanocomposites for Bottles

Improved barrier properties





Floros, 2010

Nanoparticles – Natural & Spontaneously Occurring

Natural Sources

- Volcanic dust & ash
- Forest fires
- Clay
- Viruses
- Plant and microorganism debris
- Biogenic magnetite
  - Magnetotactic bacteria
  - Protocists, arothropods
  - Birds, fish, mollusks
- Casein micelles in human breast milk

Man-made Nanoparticles

Nanoparticles – Natural and Spontaneously Occurring

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- Casein micelles in human breast milk

Man-made Nanoparticles

- Welding fumes & Abrasion dust
- Diesel emissions
- Propane engines (Zamboni machines, floor polishers & fork lifts)
- Cold catalytic converters
- Smelting
- Polymer fabrication
- Cooking (baking) & meat smoking
- Mayonnaise, Sauce Béarnaise
- Homogenized milk
  - Liposomes
  - Casein micelles (100 nm)
  - Whey proteins (3 nm)

Nanotechnology For Pet Food
(A short list of benefits)

Direct Additives

- Controlled delivery
- Prolonged & sustained release of substances
- Protection of labile substances
- Enhanced ability to transit barriers
- Enhanced solubility

Packaging

Nanotechnology For Pet Food
(A short list of benefits)

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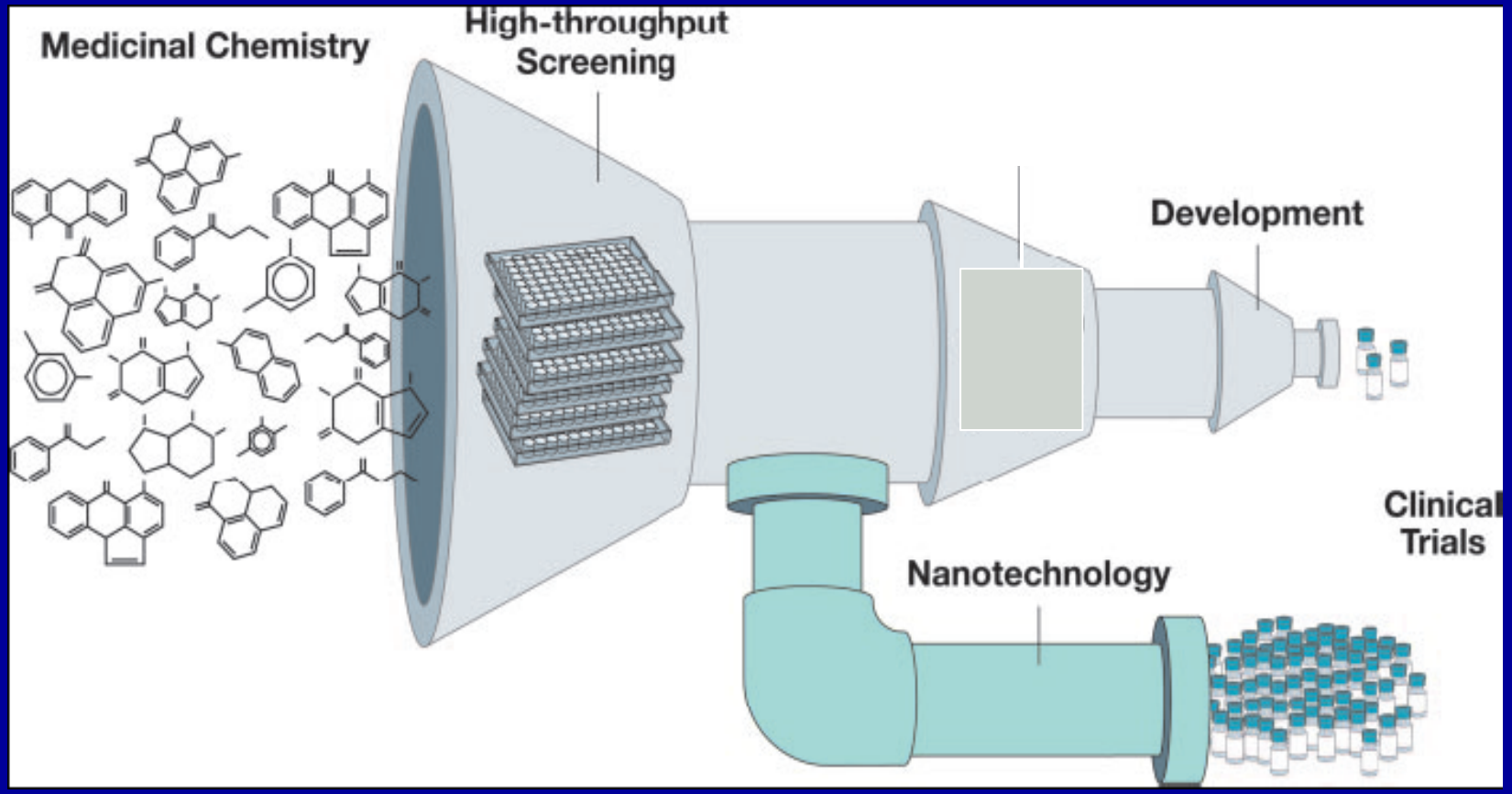
Packaging

- Enhanced barriers
  - Permeable/barrier to gases - CO_2 & O_2
  - Block UV light
  - Stronger & “self-healing” packaging
- “Smart” packaging – detection of oxidation or spoilage organisms
- Traceability
  - Decreased counterfeiting
  - Customer quality assurance
- RfID tags
- Antibacterial nanosilver for packaging and preparation areas

New Uses for Old Substances



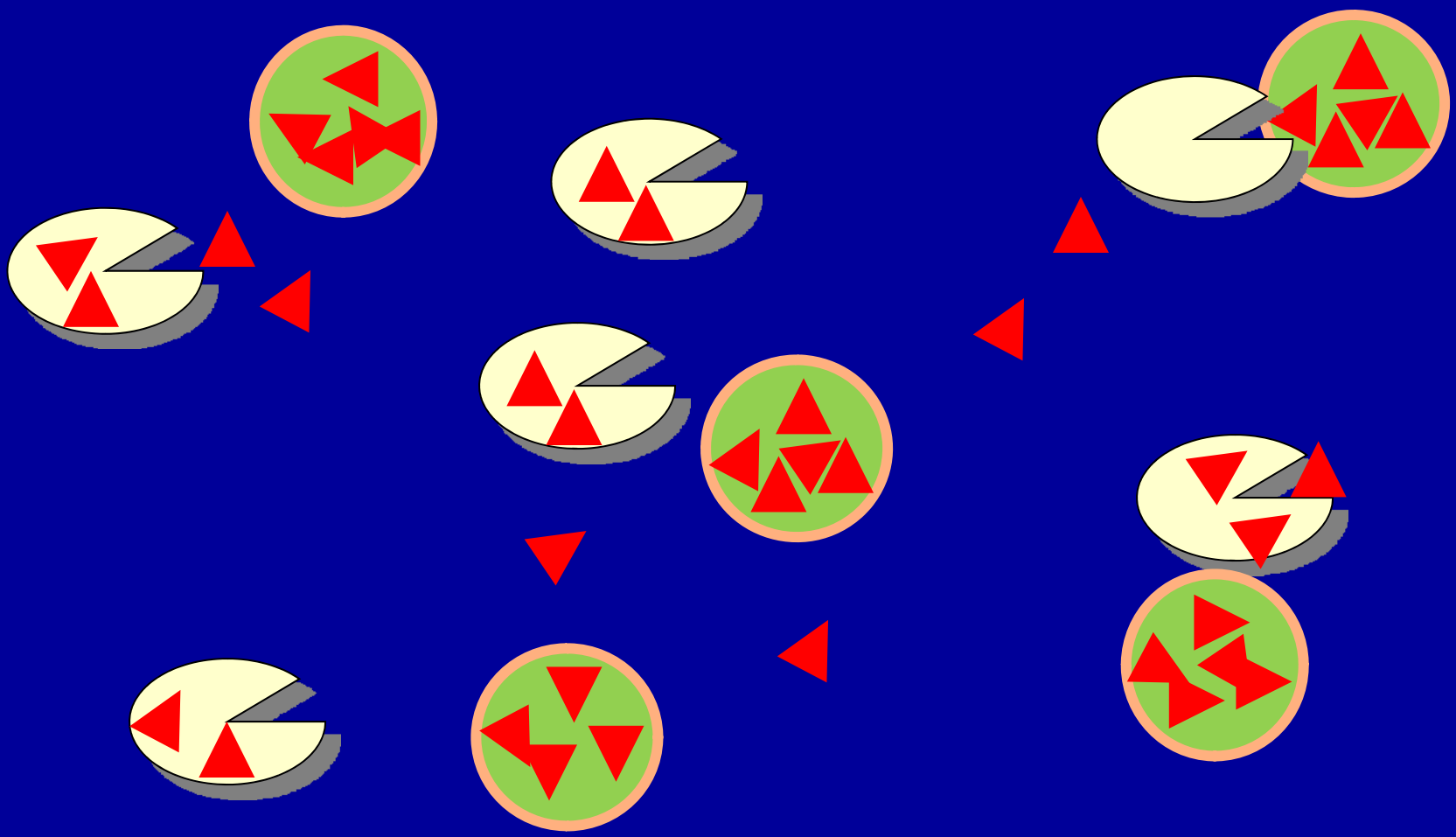
McNeil, SE. J Leukocyte Biol. 78:585, 2005

Delivery Systems – Micro (nano) Emulsions (*For delivery of oil-in-water systems)*

- Clear, transparent liquids
- Ultra-low surface tension
- Thermodynamically stable
- No phase separation over time
- Examples – lipid-soluble ingredients in aqueous systems
  - Vitamin E in beverages
  - Lipid-soluble flavor ingredients in aqueous environments

(C)? Arlington Scientific

Liposomes as Delivery Systems



Carriers may be liposomes or α-lactalbumin tubules

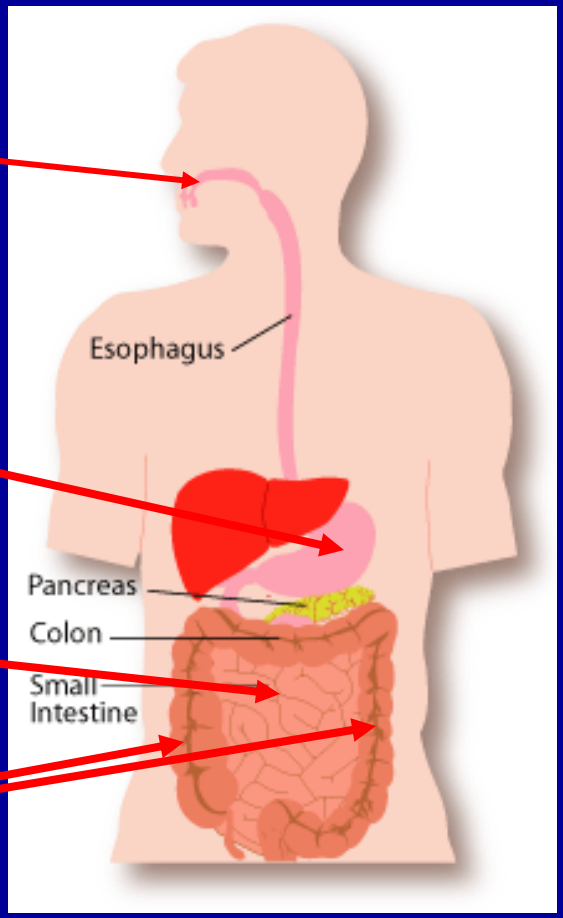
Liposomes as Delivery Systems

Mouth:
- Starches degraded by amylase

Stomach:
- Highly acidic environment
- Proteins and starches degraded by proteases & carbohydrases

Small intestine:
- Less acid environment
- Proteins, lipids and starches degraded by pancreatic enzymes

Large intestine/colon:
- More enzymes (specialized)
- Bacterial degradation

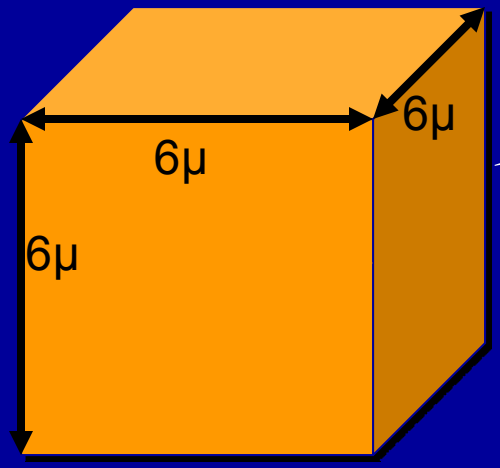


Different from Bulk Matter? Example: Effects on Solubility

Solubility can be significantly enhanced

- At macro level: intermolecular forces may prevent dissolution and may ordinarily require injection of energy into the system (e.g. heating or agitation)
- At nano level
  - Greater solubility
  - Quicker dissolution

Bigger Is Not Always Better And Smaller Is Sometimes Also Different

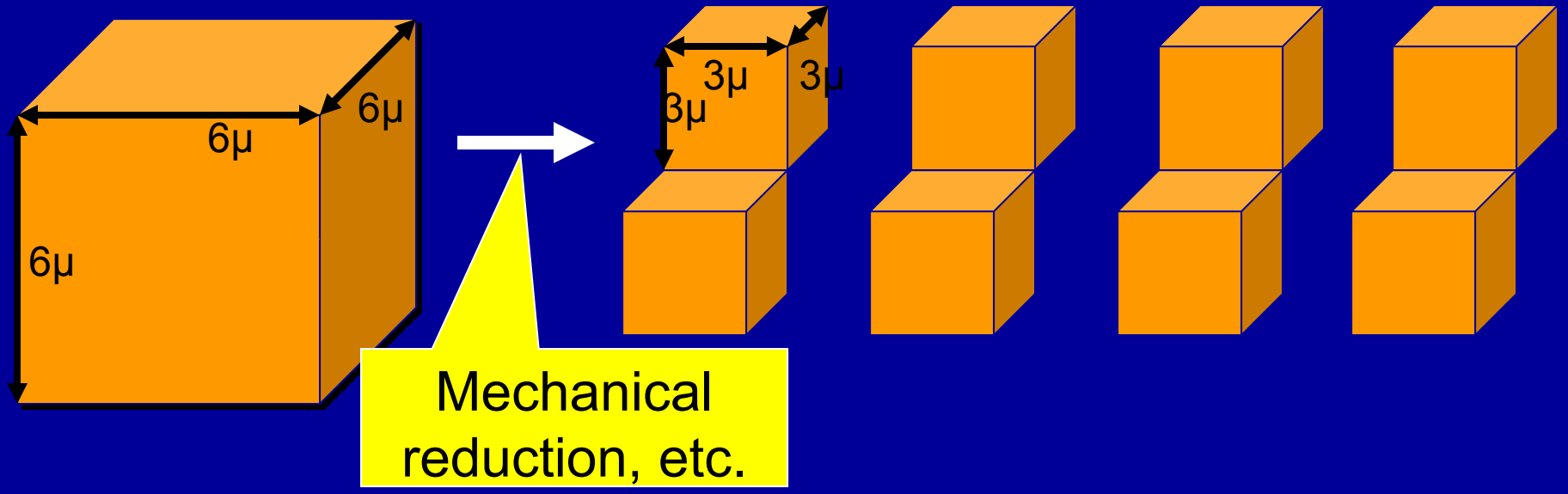


Bulk Matter -
Strong intermolecular forces
Solubility very poor

Bigger Is Not Always Better And Smaller Is Sometimes Also Different

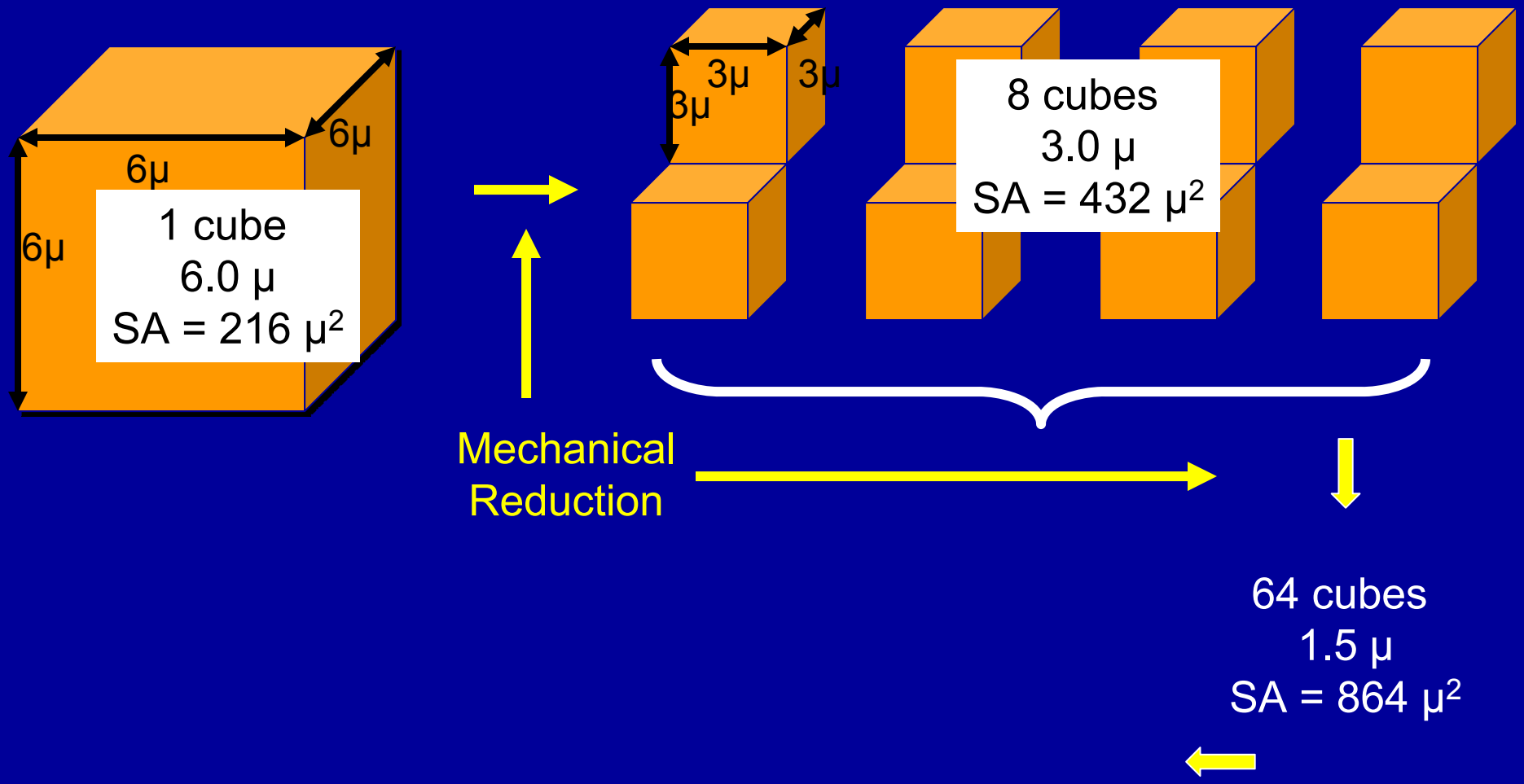
Reduction of bulk matter into smaller sub-particles.

More particles, but mass is the same.



Mechanical reduction, etc.

Effect of Particle Size on Surface Area



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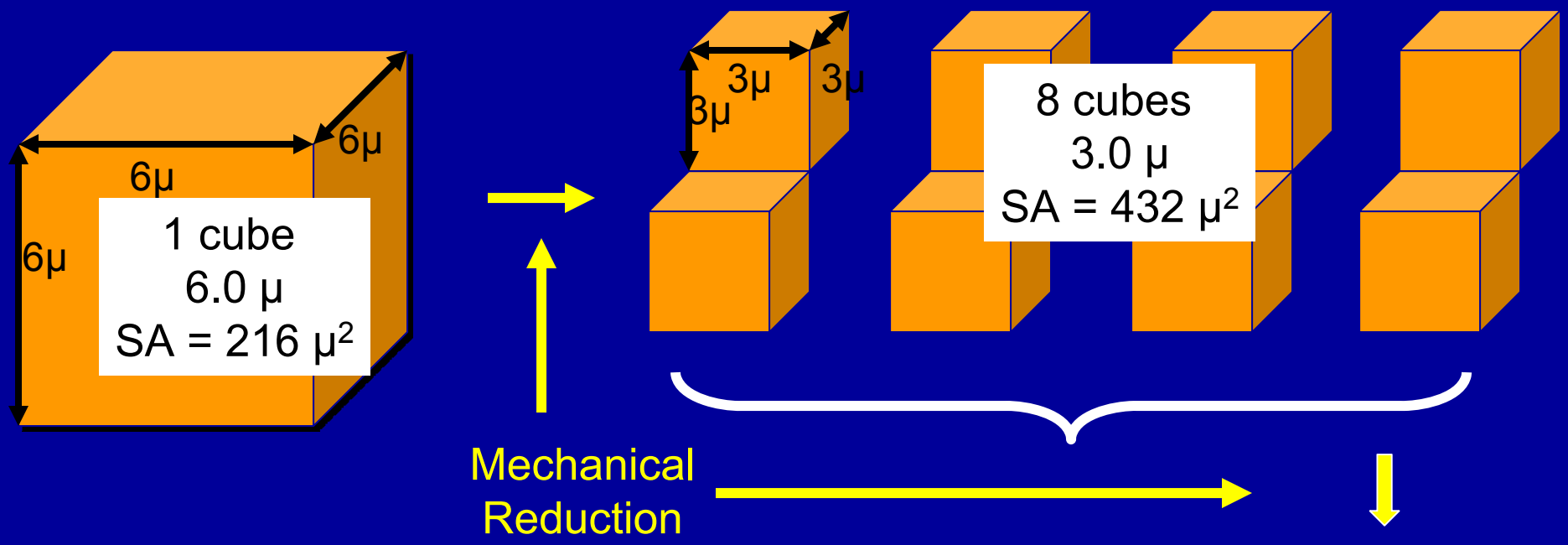

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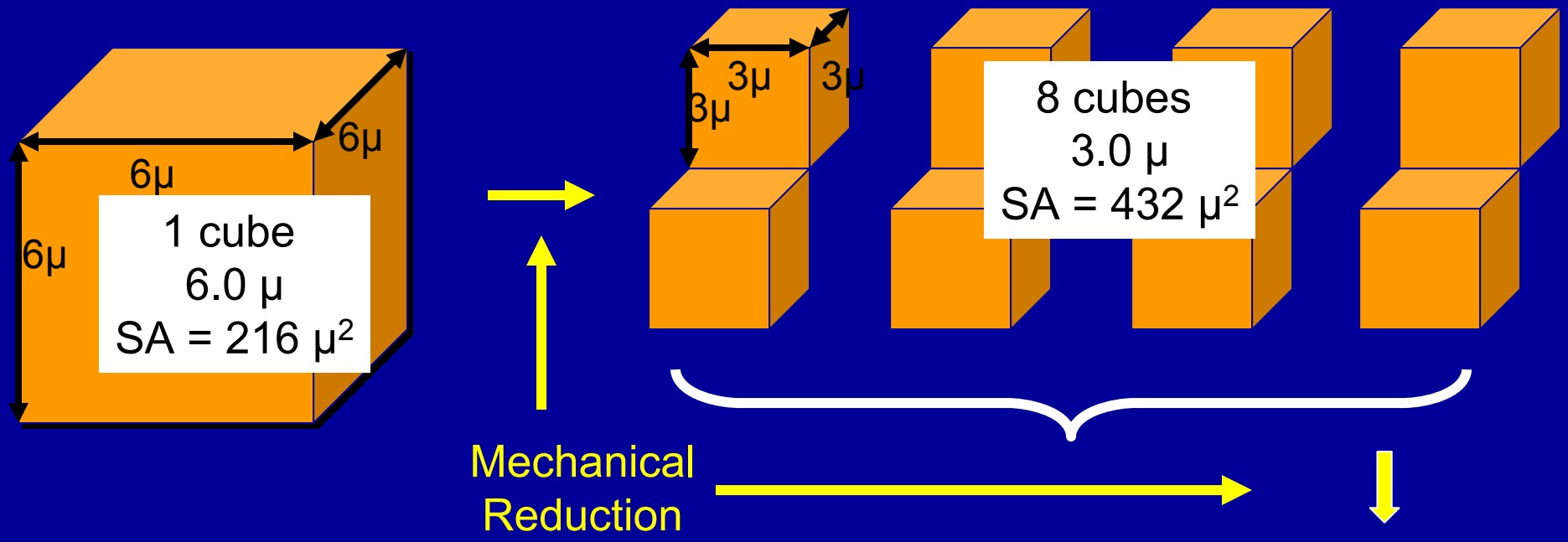

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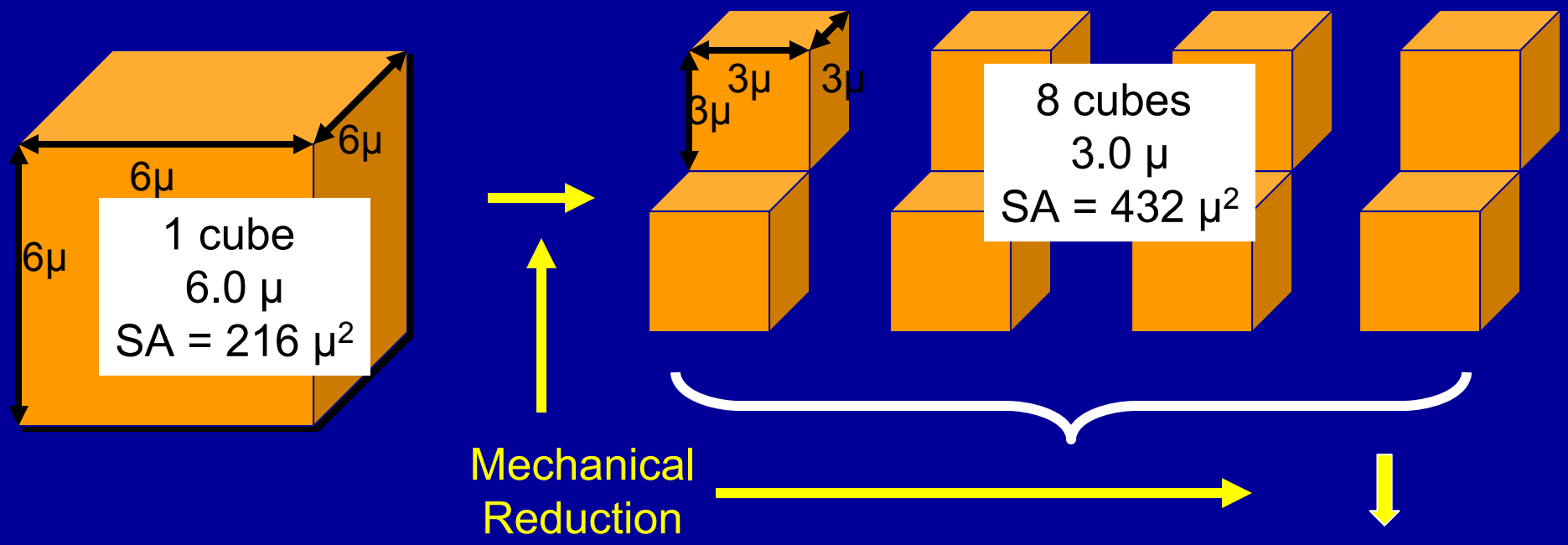

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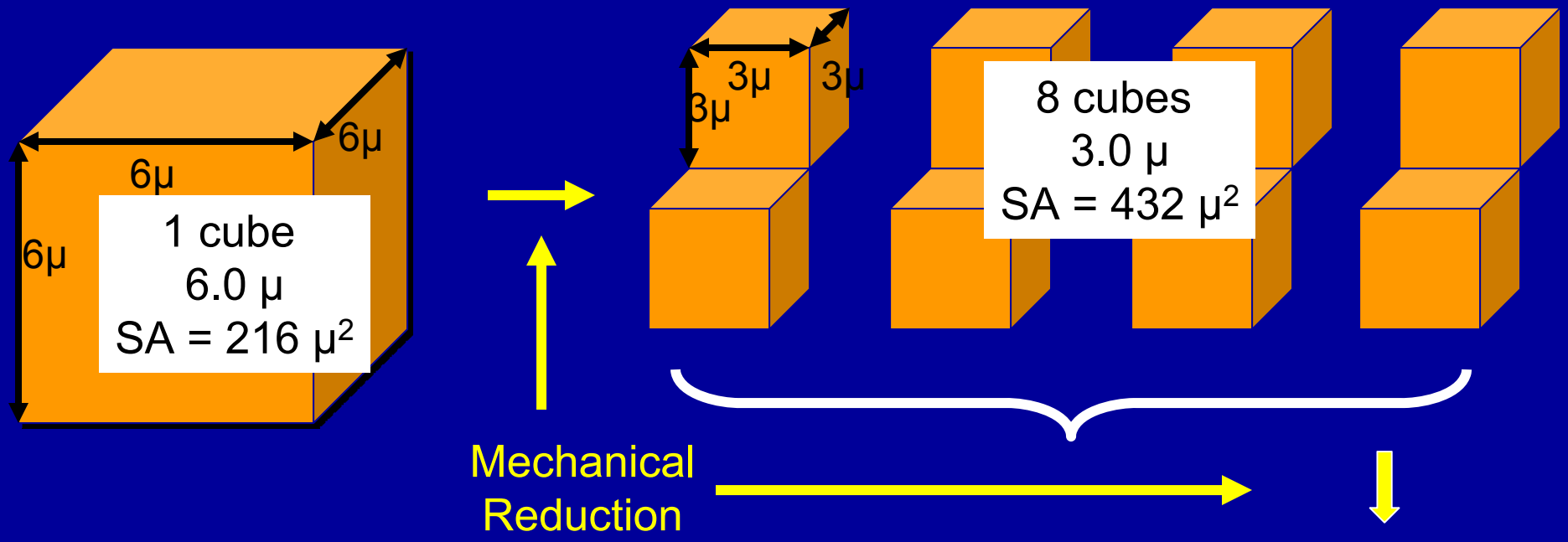

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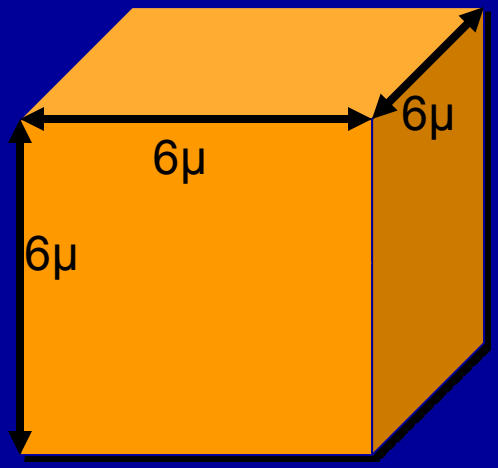
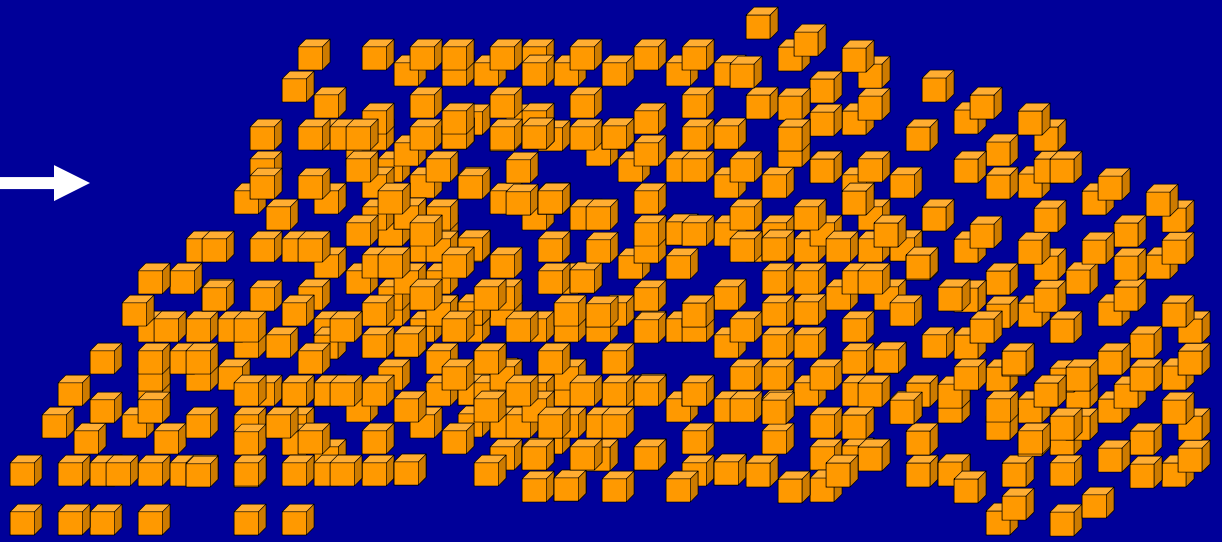


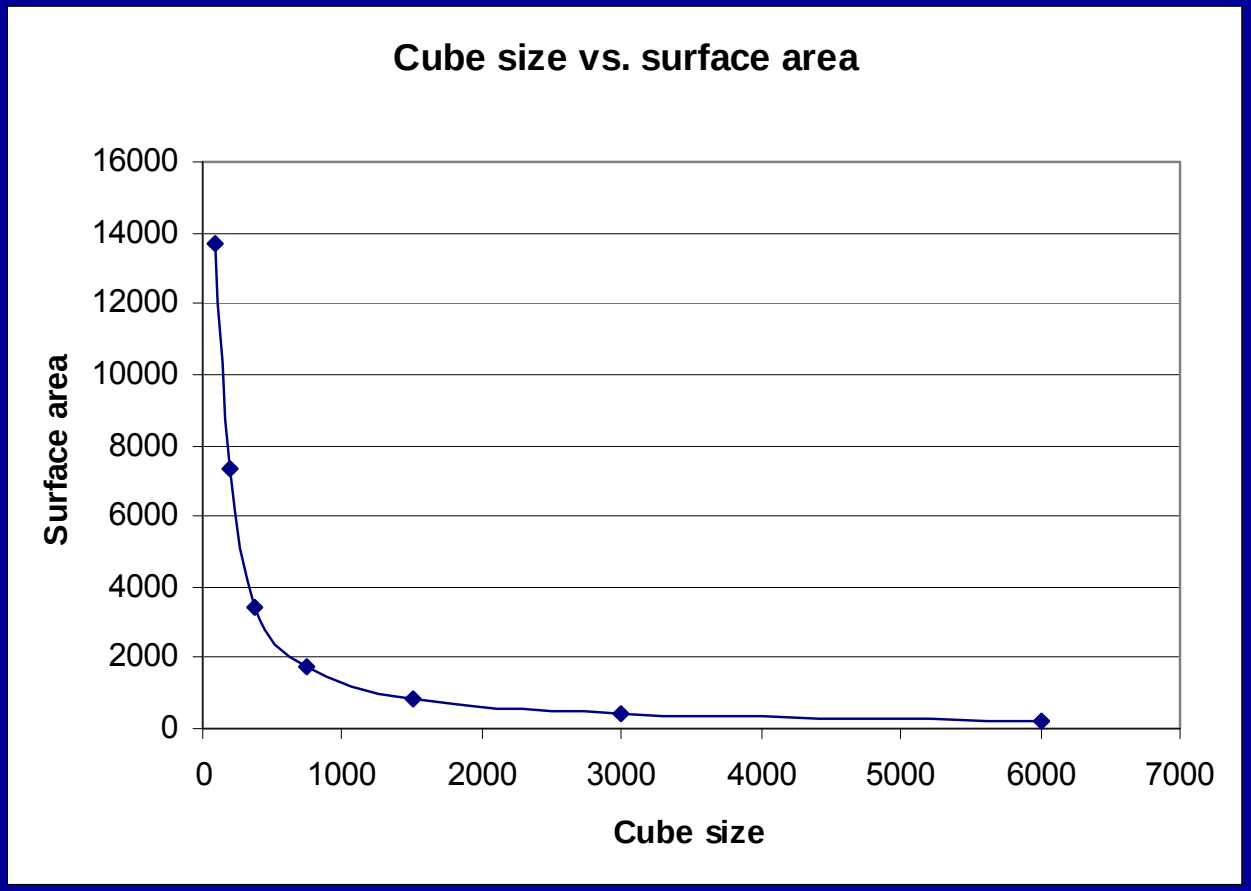


1 cube
6000 nm each side
Total surface area = 216 μ^2
Mass = 1

262,144 cubes
93 nm each side
Total surface area = 13,713 μ^2
Mass = 1

Effect of Particle Size on Surface Area

As cube dimension decreases, surface area increases exponentially.



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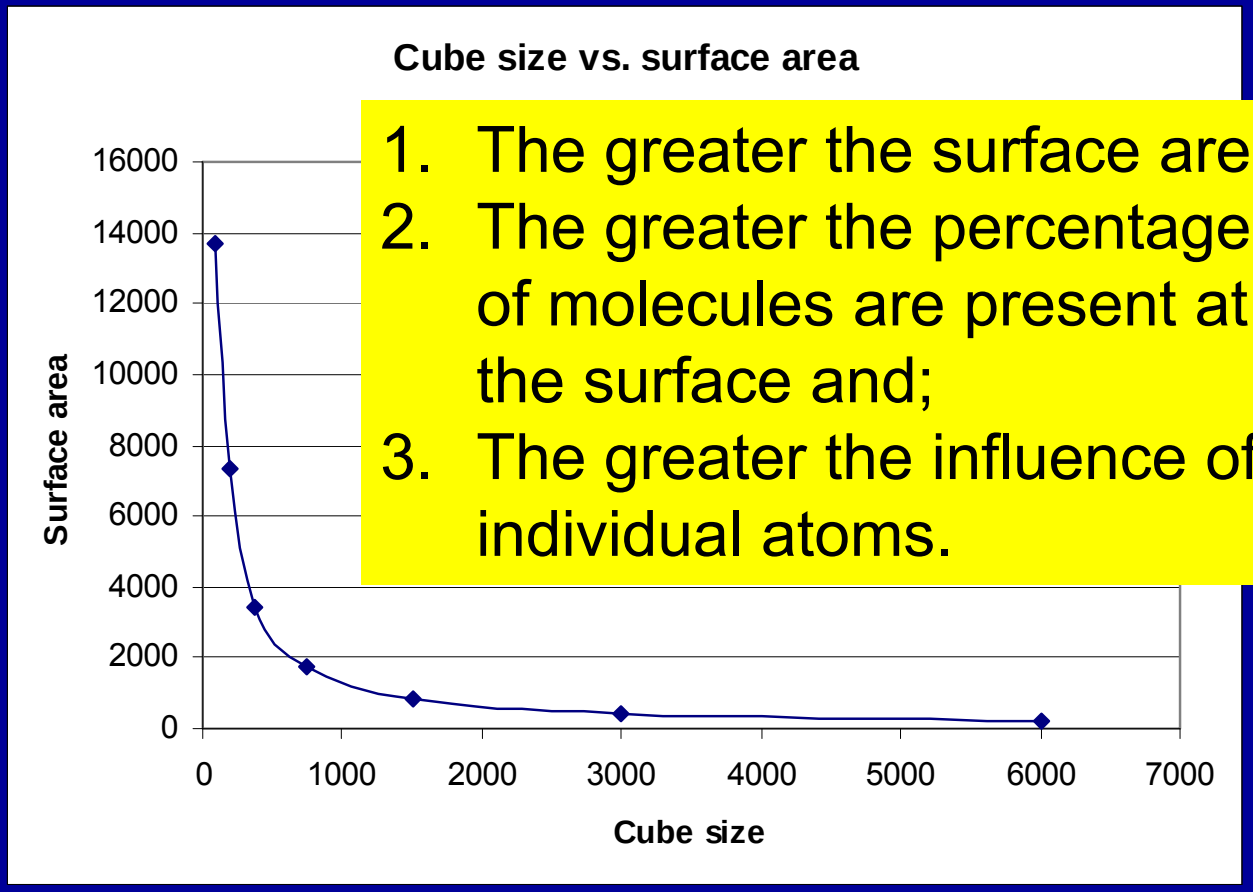
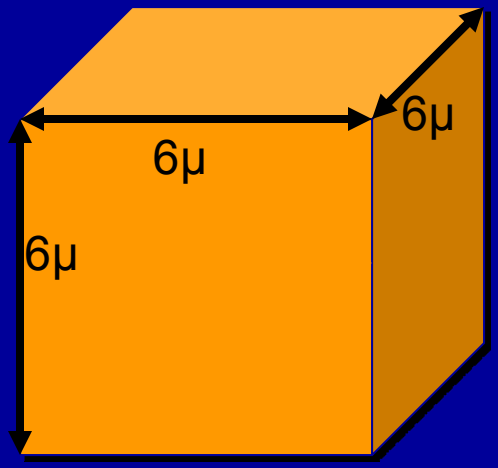
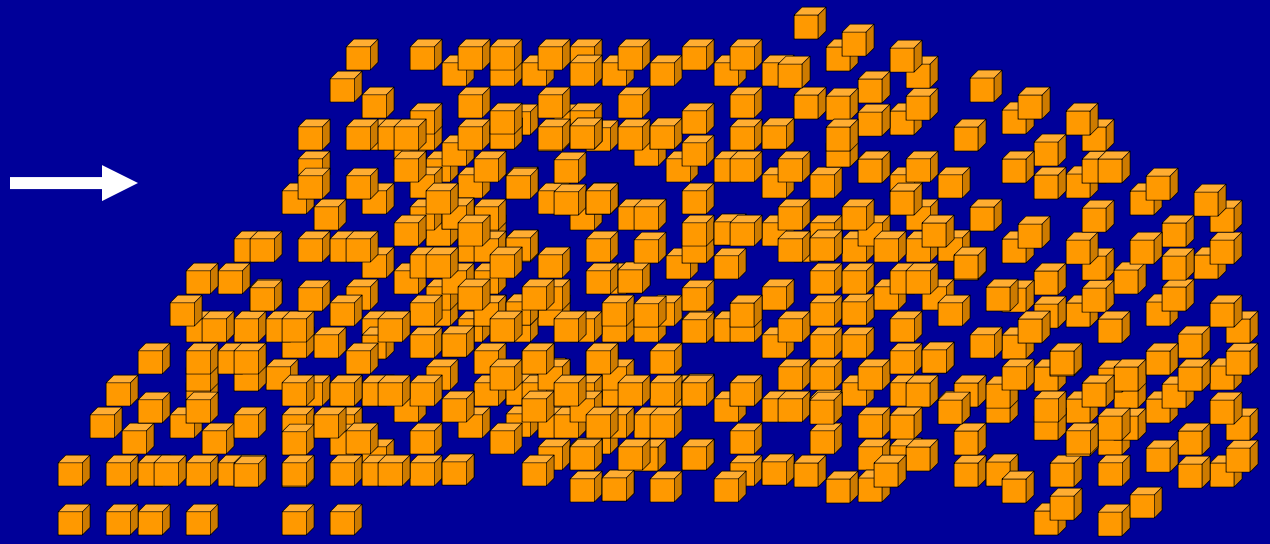


1. The greater the surface area;
2. The greater the percentage of molecules are present at the surface and;
3. The greater the influence of individual atoms.

Effect of Increased Surface Area

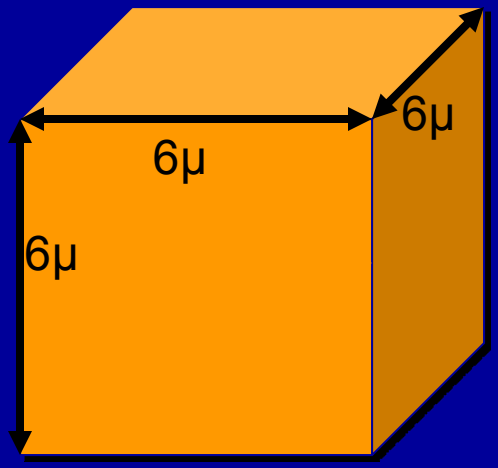
Unexpected changes

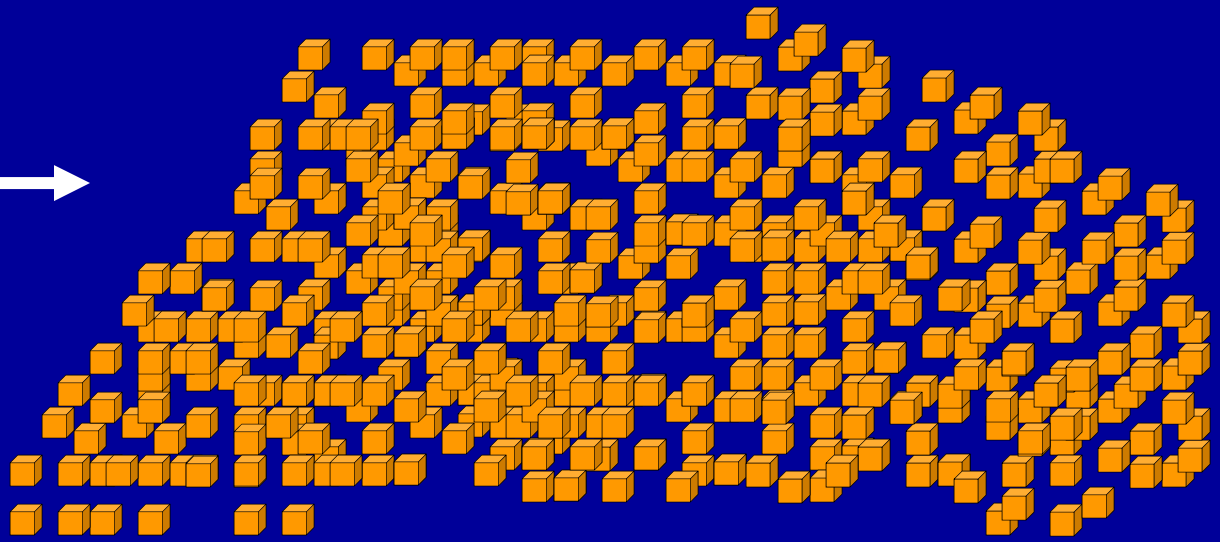




Newtonian physics → Quantum Physics

Effect of Increased Surface Area

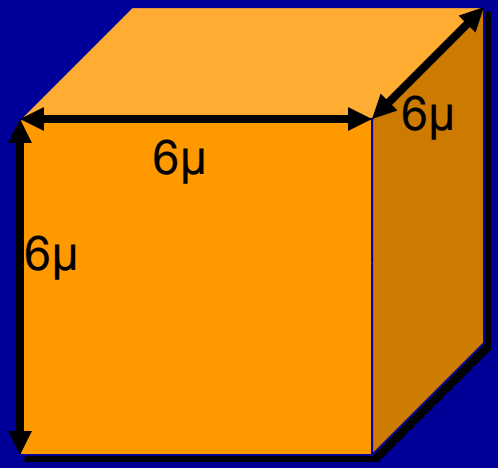


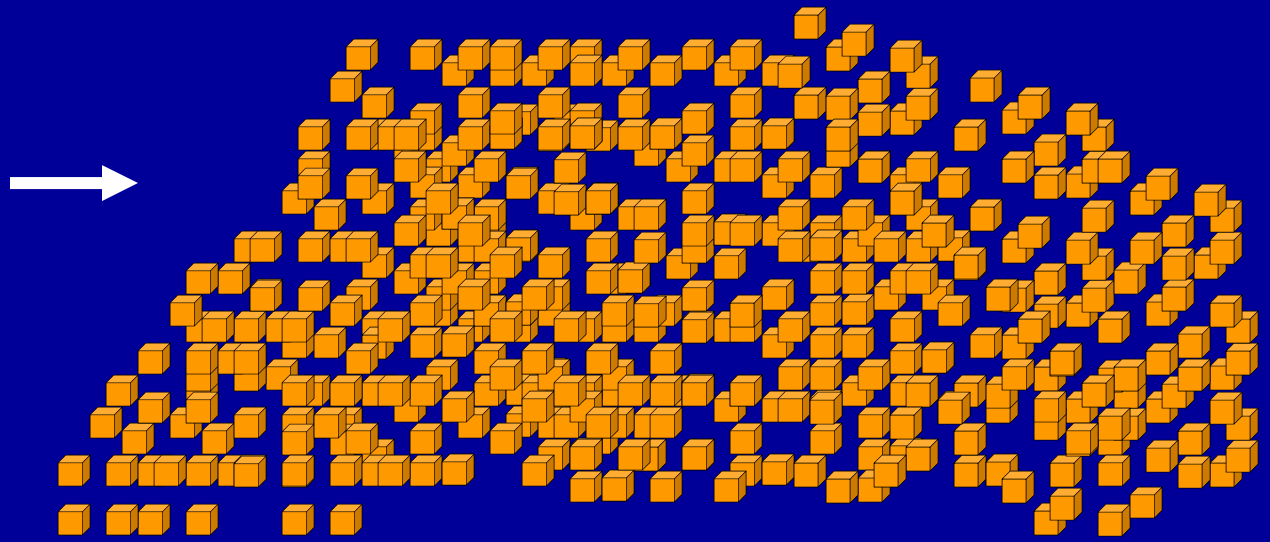


- Optical properties change
  - Zinc oxide becomes transparent
- Physical properties change
  - Melting point decreases
  - Hardness increases

- Electromagnetic properties change
  - Some metals become more magnetic
  - Some become semiconductors
- Physical properties change
  - Nanoparticles become catalysts
- Gravitational effects change
  - Gravitational effects negligible

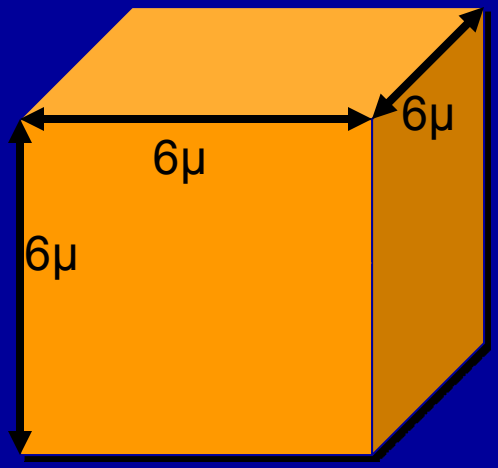
Effect of Increased Surface Area

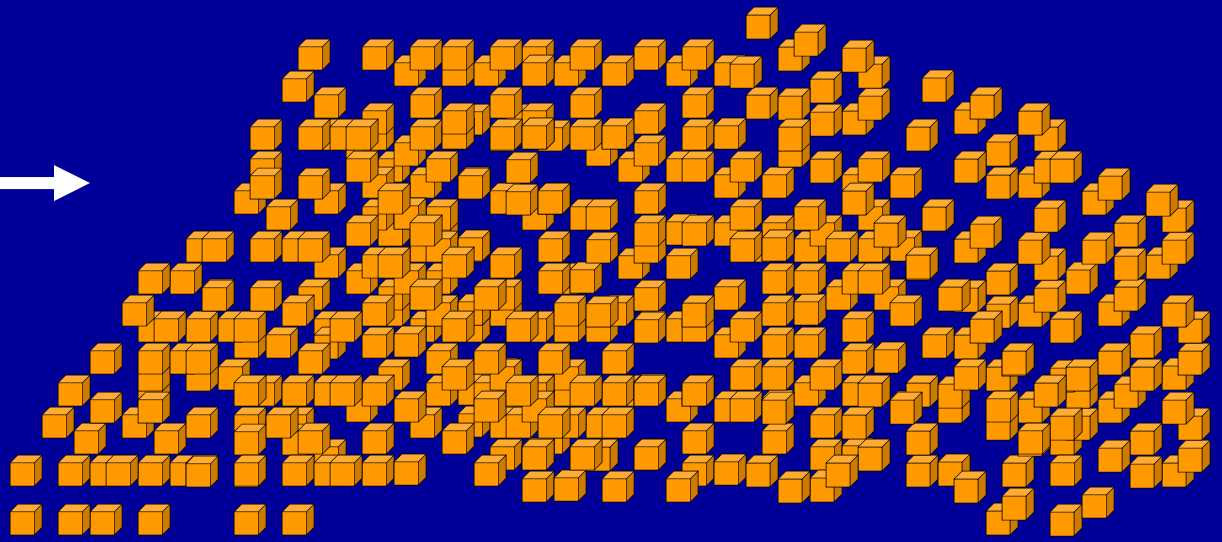




Insoluble → Soluble

Effect of Increased Surface Area





Insoluble → Soluble

Solubility changes → Absorption & biokinetic changes

Nanotechnology Fundamental Differences

Change Physico-Chemical Properties

The importance of this to you is:

- because there is a *difference in physico-chemical properties* at the nanolevel,
- it is only logical to assume that a *different biological activity* may result.

Mediators of Activity of NSPs

(Includes all solid particles and liposomes, such as fat or lactalbumin envelopes)

- Size
  - (& size distribution)
- Shape
  - Spheres
  - Wires
  - Tubules
  - Plates
- Chemical composition
  - Coatings
  - Impurities (esp. Fe, furan)

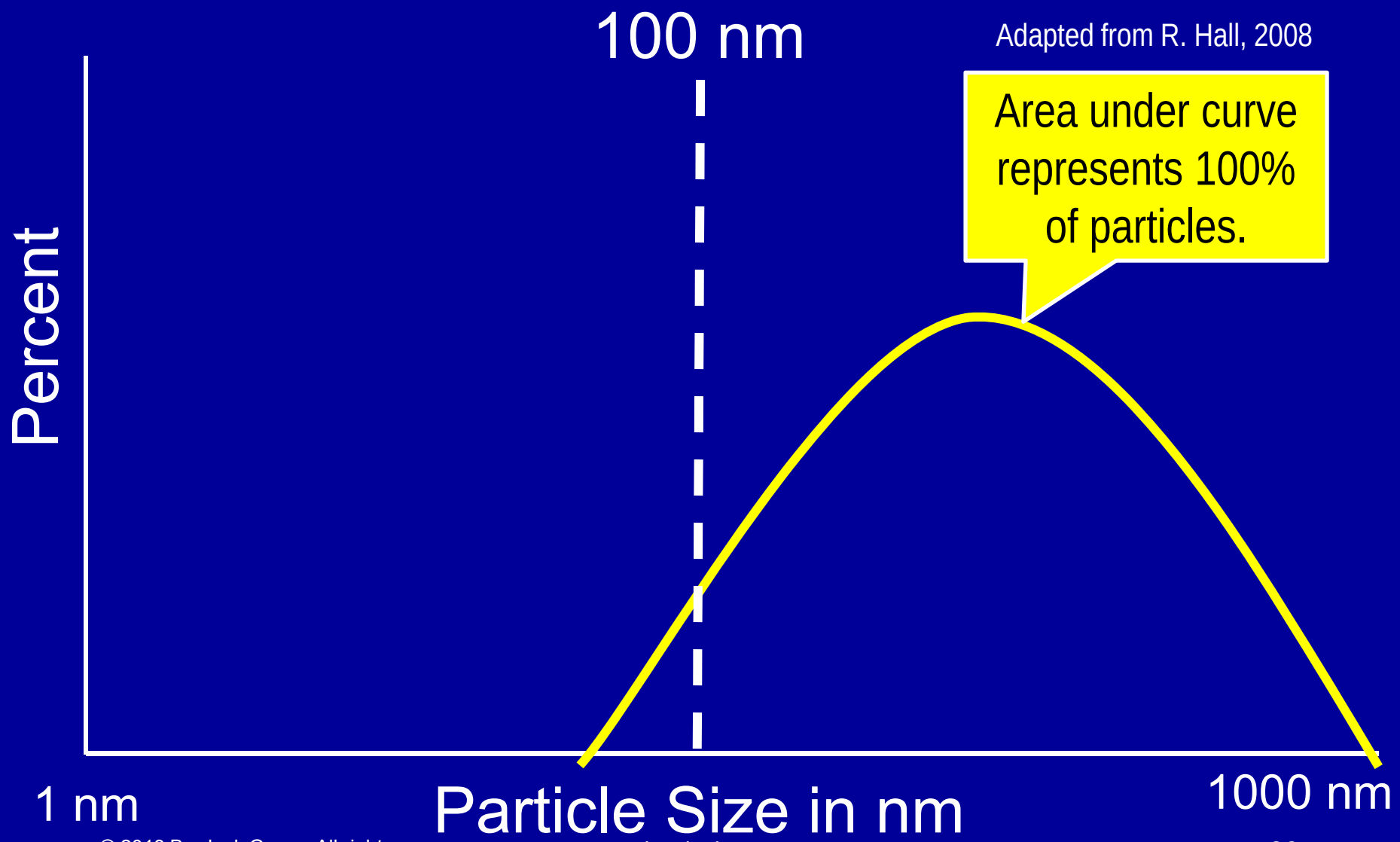
- Density
- Agglomeration state
- Solubility
- Porosity
- Surface charge
- Roughness & morphology
- Crystal structure
- Magnetic properties
- Surface defects (carbon nanotubes)**

NSP = Nano Sized Particle

Oberdorster et al., Particle and Fibre Tox 2005

***Muller et al Chem Res Toxicol, 2008*

Importance of Particle Size Distribution



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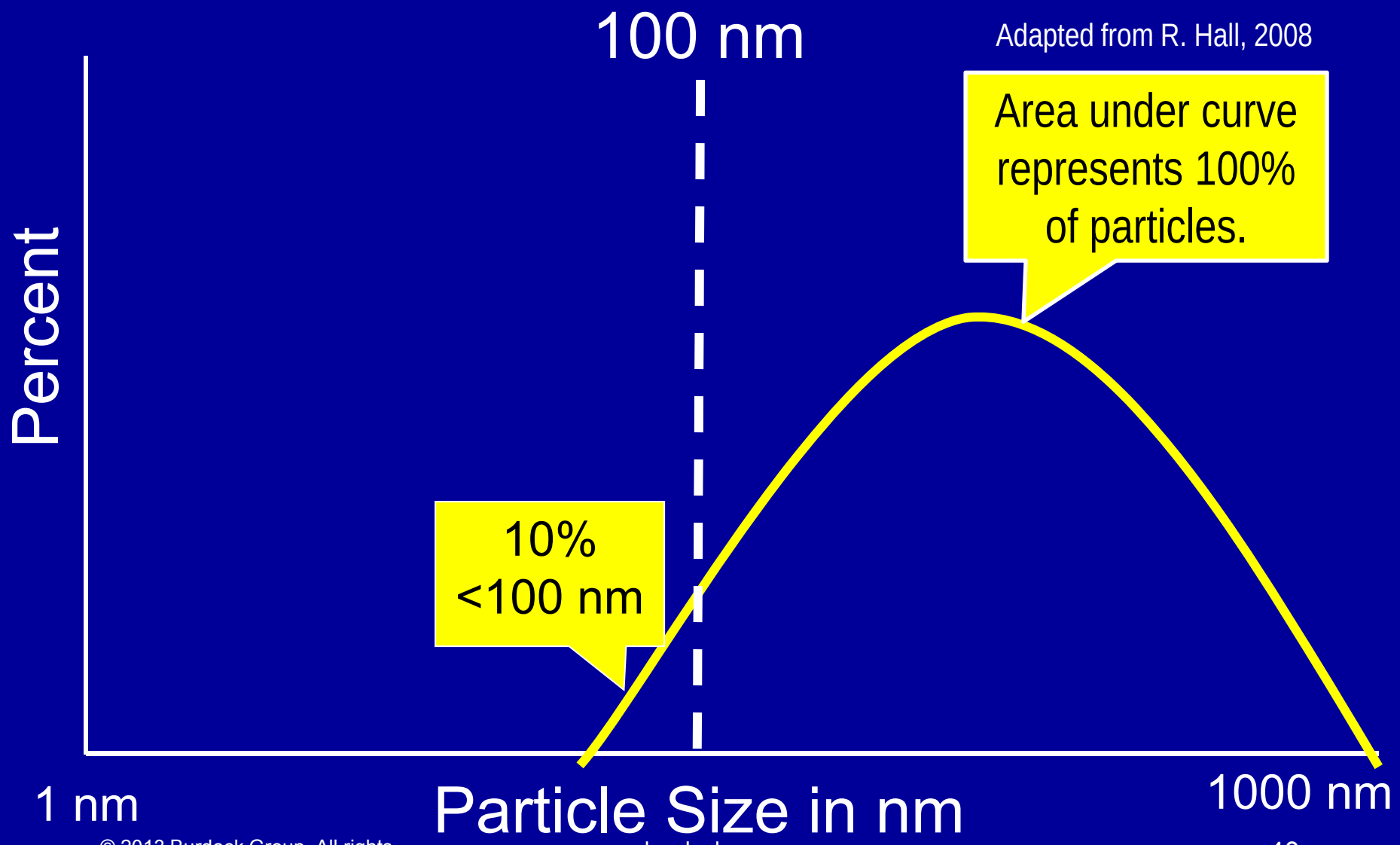

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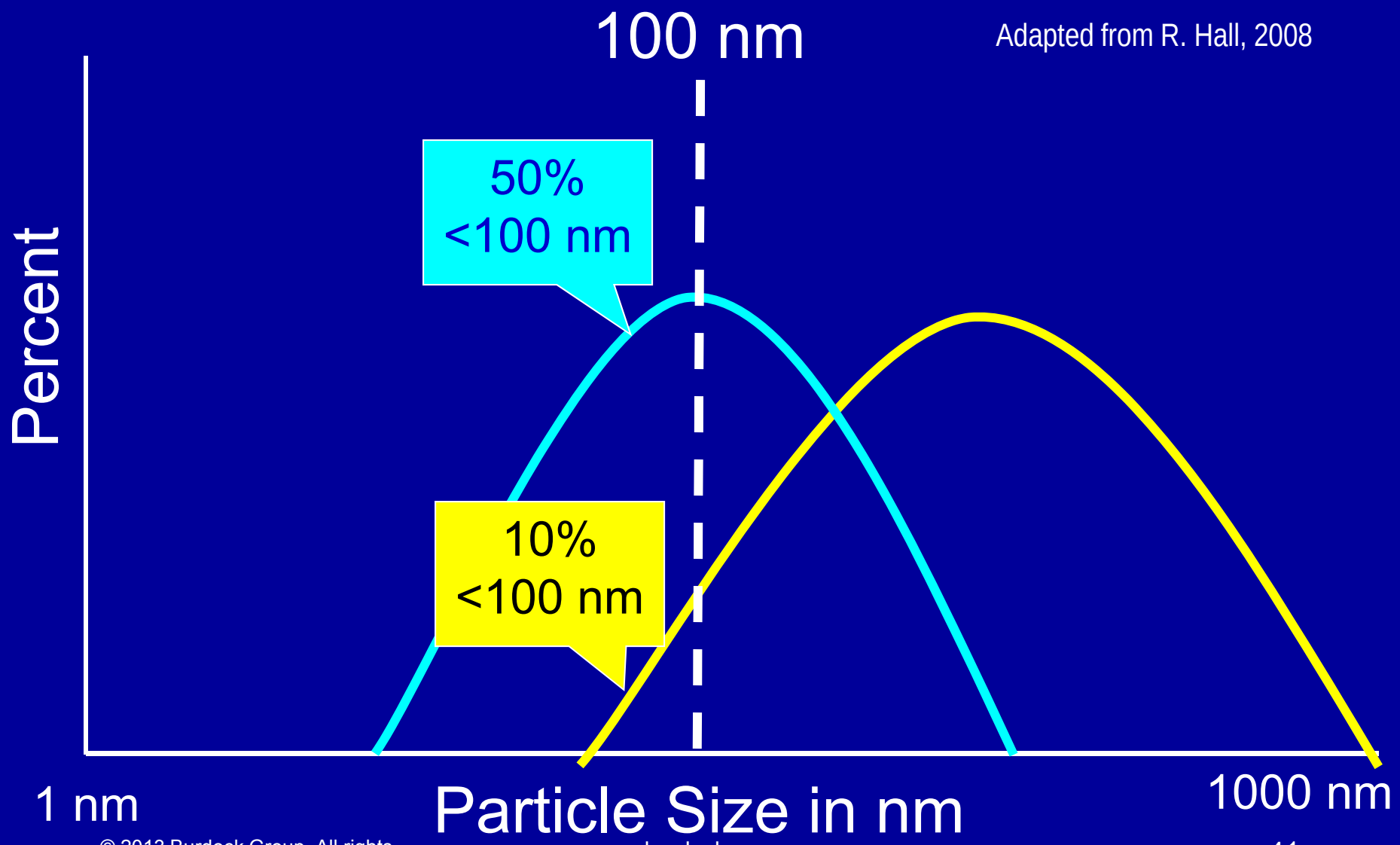

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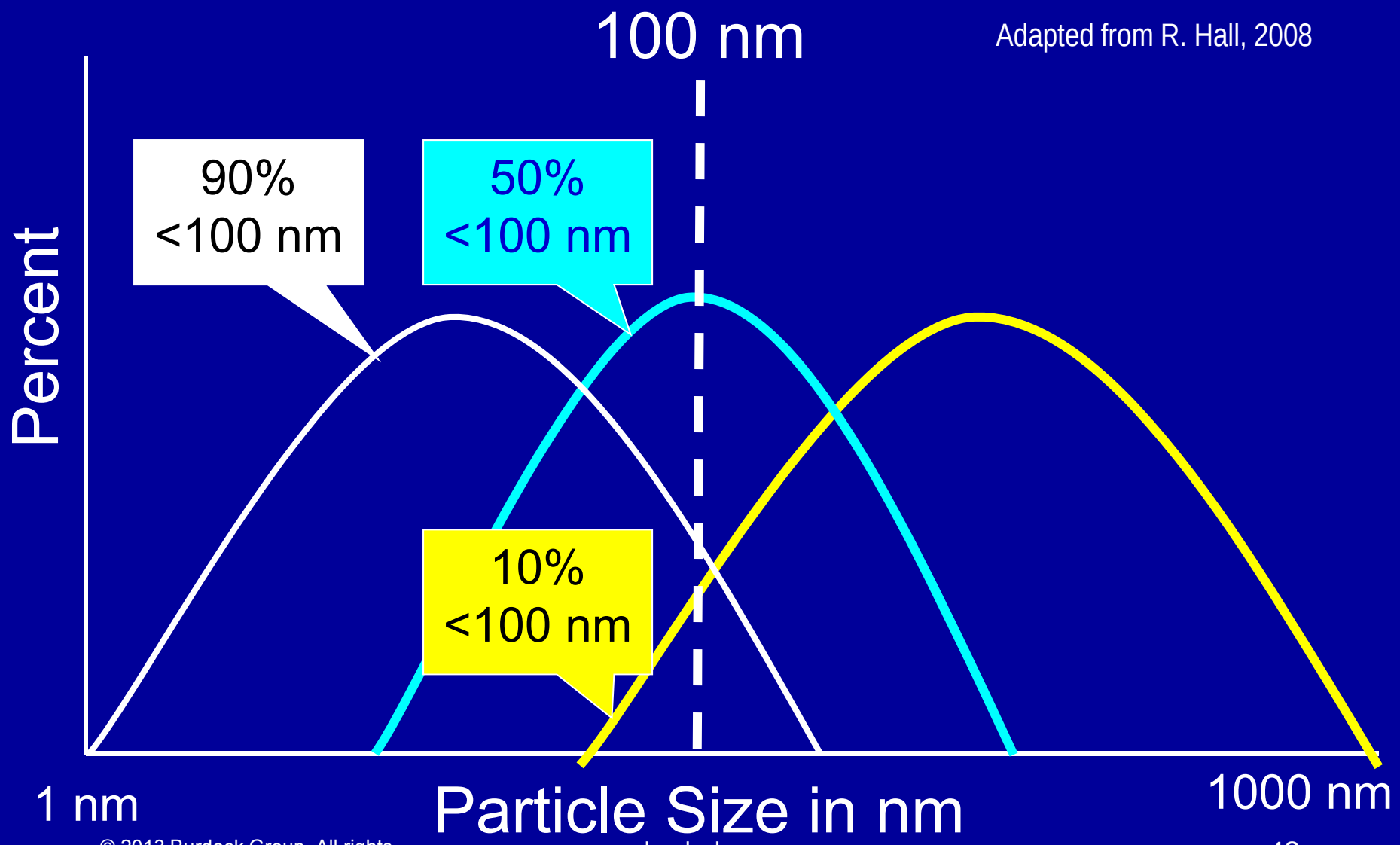


Principal Mediators of Toxicity of NSPs? Shape?

Shape often determines how an organism will mount a response to the particle

- Asbestos fiber dimension importance in mesothelioma
- Single-wall nanotubes are more toxic than multi-wall nanotubes

Principal Mediators of Toxicity of NSPs? Electrical Charge

Charge of particle may determine its toxicity.

- Nanoparticle surface charges alter blood–brain barrier integrity and permeability (cations disrupt BBB)

  Lockman *et al.* (2004). Journal of Drug Targeting 12:635-641.

- Altering charge may reduce or increase toxicity

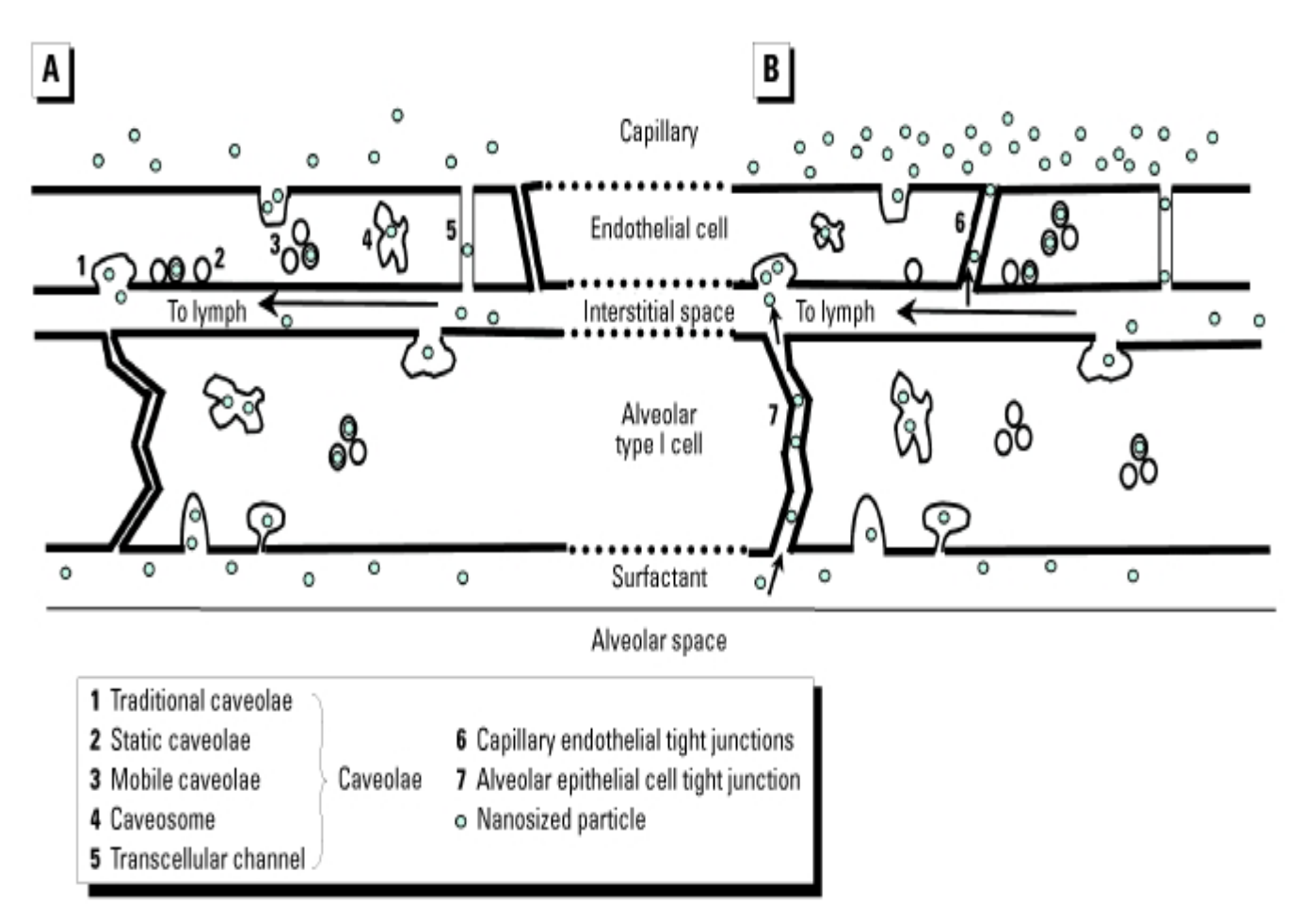
  Derfus et al. (2004). *Nano Lett.* 4:11-18.

Principal Mediators of Toxicity of NSPs? Size?

- Absorption
- Translocation
- Decreased clearance rate
- Traditional barriers (e.g., BBB) may no longer be valid.

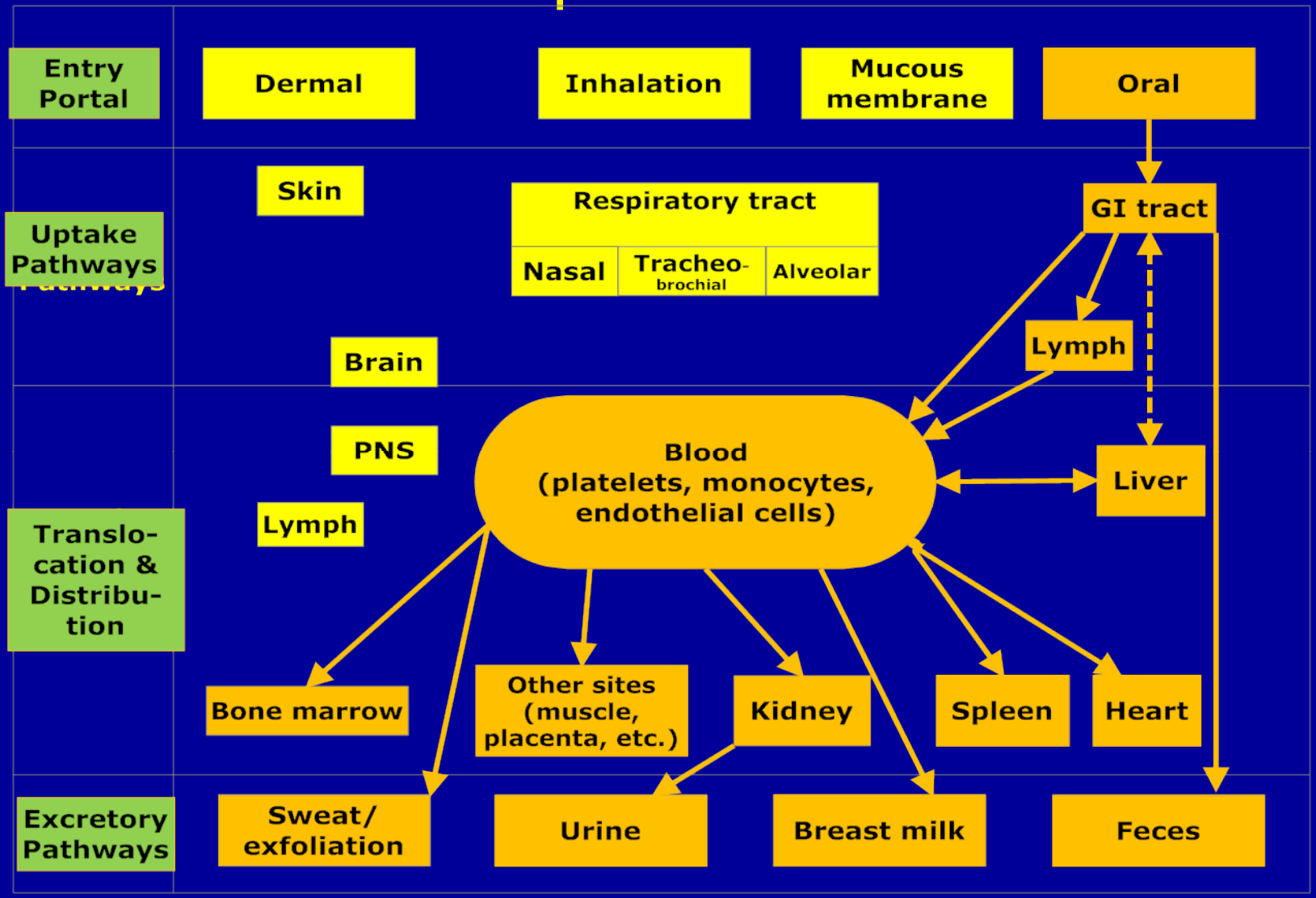
As particles become smaller, the ease of absorption and distribution increases.

Absorption of NSPs Greatly Enhanced



Oberdorster et al EHP, 2005

Oral Absorption & Distribution



Principal Mediators of Toxicity of NSPs? Can Size Amplify Intrinsic Toxicity?

The smaller the particle, the more surface area per unit mass.

Therefore, if the substance is intrinsically toxic, then the toxicity of the substance is emphasized because of its great surface area.

As particles become smaller, the likelihood of causing harm increases.

Principal Mediators of NSP Toxicity
The dose makes the poison

Traditional thinking: effects are correlated to the mass of the agent to which the individual is exposed – *the dose makes the poison.*

Principal Mediators of Toxicity of NSPs? Can Size Change the Intrinsic Toxicity?

Traditional thinking: effects are correlated to the mass of the agent to which the individual is exposed – *the dose makes the poison.*

Nanotoxicology: (given the same mass) the number of particles and the resulting surface area determine the effect – *the dose (as a function of surface area) makes the poison.*

Regulation is often Risk-based, BUT Traditional Risk Assessments May No Longer Apply for NSPs

1. Intrinsic toxicity (hazard) may be enhanced as particles become smaller.
2. Hazard characterization changes:
   a. As particles become smaller, the likelihood of absorption and distribution increases
   b. Shape often determines how an organism will mount a response to the particle
3. Exposure is no longer simply dose, but dose as a function of particle size.

Traditional Risk Assessments May No Longer Apply

What Does This Mean for Feed Ingredient Safety?

Because traditional risk assessments may no longer apply, then:

Historical assumptions of safety & previous approvals - may no longer be valid!

You may have breached the "may render" standard or "unfit" standard (FFDCA §402) where the substance is no longer "reasonably certainty of safety".

Why is it no longer safe?

- Accelerated rates of absorption (bolus dose)
- Susceptible population subgroups
- Create new allergens
- New toxic sequelae observed
  - Previously protected environments
  - New interactions
- Oxidative stress – Reactive Oxygen Species (ROS)

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"…induction of oxidative stress is a major mechanism of nanoparticle effects…"
Oberdorster et al, 2007

Can Nanotechnology be Regulated?

"A particular advantage of liposomes is that they consist solely of naturally-occurring constituents, potentially reducing or eliminating regulatory hurdles that may prevent their application to food systems."

Critical. Rev. Food Sci & Nut. 45:587, 2005

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Man-made Nanoparticles

- Cooking (baking) & meat smoking
- Mayonnaise, Sauce Béarnaise
- Following homogenization of milk
  - Liposomes
  - Casein micelles (100 nm)
  - Whey proteins (3 nm)

Can Nanotechnology be Regulated?

"A particular advantage of liposomes is that they consist solely of naturally-occurring constituents, potentially reducing or eliminating regulatory hurdles that may prevent their application to food systems."

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The occurrence of naturally forming liposomes is not sufficient rationale for creating them for use in animal feed.

Man-made Nanoparticles

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FDA's authority is very clear

"A food shall be deemed to be adulterated if it bears or contains any poisonous or deleterious substance which may render it injurious to health…"

FFDCA §402(a)(1)

Can Nanotechnology be Regulated? Regulation in other venues

- Food irradiation – a food additive
  - Still contentious while people die from hemmorrhagic *E. coli*
- Genetically modified organisms – requires special consults with FDA
  - Mandatory labeling in Europe
- Dietary Supplements & Health claims
  - A 50 year battle
- Endocrine disrupting chemicals
  - 87,000 chemicals to be tested with no valid test in sight

What is Required to Show Safety?

- National Cancer Institute
  - Nanotechnology Characterization Laboratory

- FDA
  - Presently on a case-by-case basis
  - Guidelines issued April, 2012
    - http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm300661.htm

FDA Requirements for Particle Size Already in Place

Color Additive Petitions

- Section III.A. Identity:
  - "…If the particle size is important for the additive to achieve its intended technical effect,
  - "…data on the size (average and distribution), shape, surface area (average and distribution), surface charge (zeta potential), and morphology of the particles, as well as any other size-dependent properties(e.g., agglomeration, aggregation, dispersion) should be included, as appropriate.

FDA Requirements for Particle Size Already in Place

Food Additive Petition

- Section III.C. Specifications for Identity and Purity:
  - "…Parameters related to the particle size, shape, and surface properties of the food additive, as appropriate, if particle size is important for the identity and functionality of the additive."
- Section III.E. Intended Technical Effect and Use:
  - "...A clear statement of the intended technical effect(s) of the additive in food. If technical effect of the additive is related to particle size, the statement should explain how size-dependent properties of the additive affect functionality (e.g., solubility, viscosity, stability, antibacterial properties, antioxidant properties)."

What do you really need?

- Must characterize your NSP
- Otherwise, standardized safety testing protocols are already available
  - Do not “over test” out of a sense of over-conservatism
  - Do not “test yourself out of a product”
- Get *professional* advice on exactly what is needed to for a determination of safety
  - Vicarious liability

Pragmatic Path Forward

- *…the approaches and study protocols for routine toxicological characterization of chemicals are sufficiently robust to provide meaningful characterization of nanoscale materials…*

(NTP/NIEHS, 2004)

- No new testing protocols needed
- Conventional testing works

Is Nanotechnology Important?

"Nanotechnology has been compared to the industrial revolution in terms of its impact on society…"

J. Clarence Davies, 2008
Testimony before Congress

Nanotechnology is a "disruptive" technology –
A "game changer" technology

Nanotechnology:
Fundamental Changes Occur

"It's like you can shrink a cat and keep shrinking it, and then at some point, all at once it turns into a dog."

National Geographic, June 2006

Nanotechnology:
Fundamental Changes Occur

"It's like you can shrink a cat and keep shrinking it, and then at some point, all at once it turns into a dog."

Is this the dog that will turn around and bite us?

National Geographic, June 2006

Nanotechnology & Pet Food: What is it? Defining it's importance to you.

George A. Burdock, Ph.D.

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Fellow, American College of Nutrition

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