ความปลอดภัยทางนาโนเทคโนโลยี

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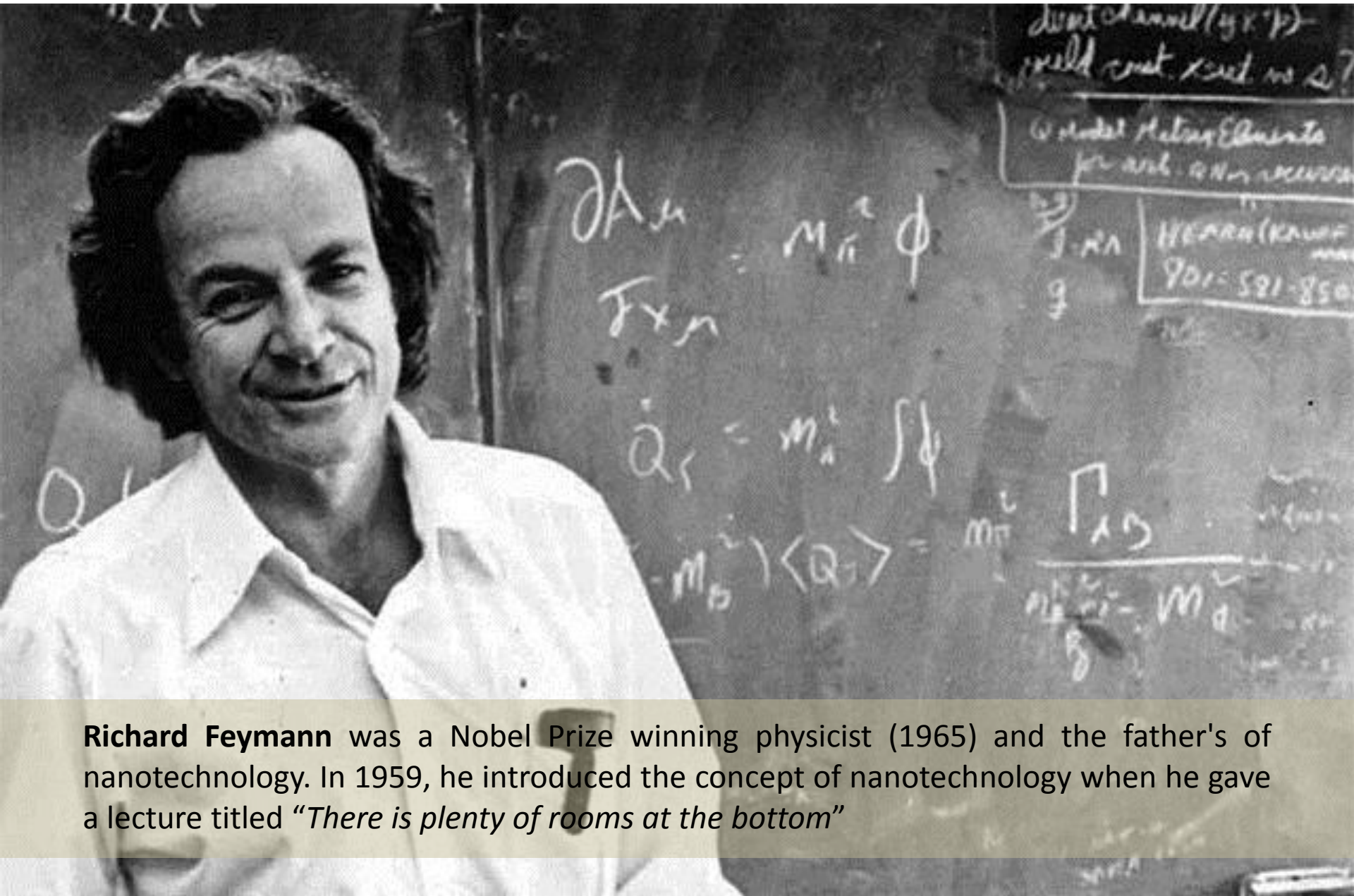
ศูนย์ความปลอดภัย อาชีวอนามัยและสิ่งแวดล้อม จุฬาลงกรณ์มหาวิทยาลัย
ณ ห้องบานเย็น ชั้น 15 อาคารมหาวชิรุณหิศ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
วันศุกร์ที่ 24 พฤษภาคม 2562

Outline

- **Introduction:** Nanotechnology and Nanomaterial Basic
- **Exposure to Nanomaterials**
- **Case-studies: Biological Effects of ENMs**
- **Toxicity Testing and Caveats in Nanotoxicology Assays**
- **Ecotoxicology of ENMs**
- **Working Safely with ENMs**
- **Regulatory Context**

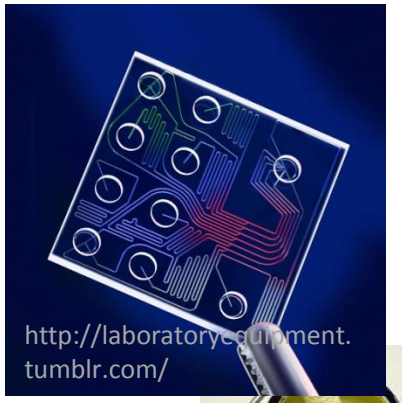
Nanotechnology



Richard Feymann was a Nobel Prize winning physicist (1965) and the father's of nanotechnology. In 1959, he introduced the concept of nanotechnology when he gave a lecture titled "*There is plenty of rooms at the bottom*"

Nanotechnology: A promising future

- Cancer therapy
- Diagnostic
- Tissue engineering
- Sensor
- Aerospace
- Protective equipments
- Electronics & wiring
- Energy
- Advanced materials in consumer products
- etc.



Nanomaterials

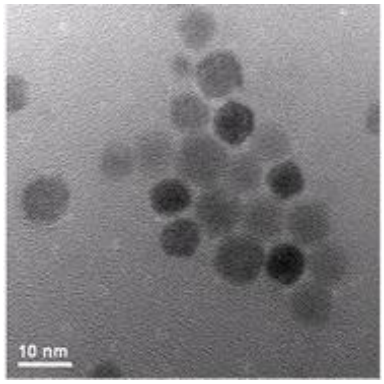
Old

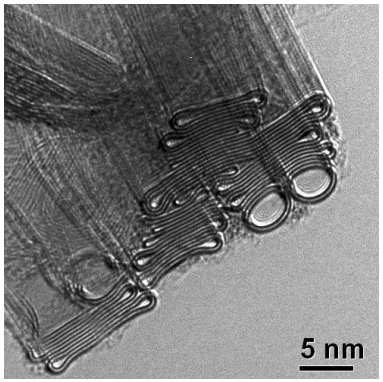
- Non-engineered nanomaterials
- Ultra fine particles (UFPs)

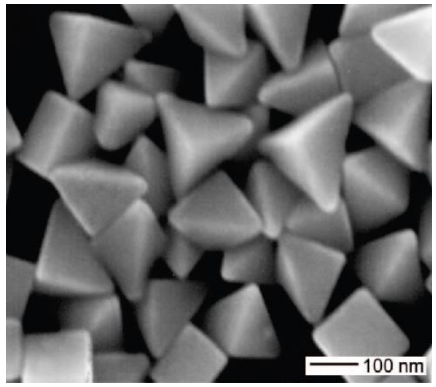


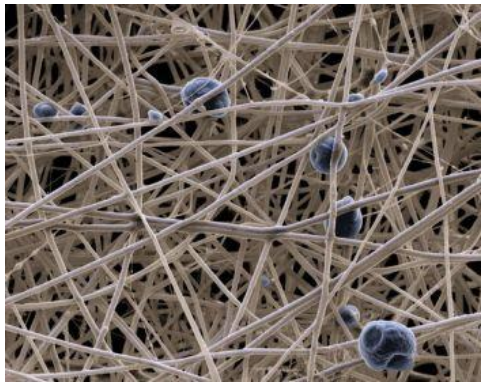

New

- Engineered nanomaterials (ENMs)







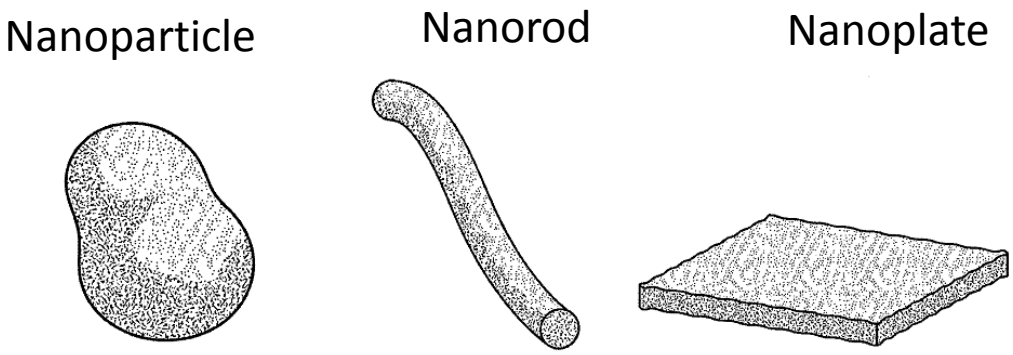


Definitions

ISO/TS 27687:2008 (revised by ISO/TS 80004-2:2015)

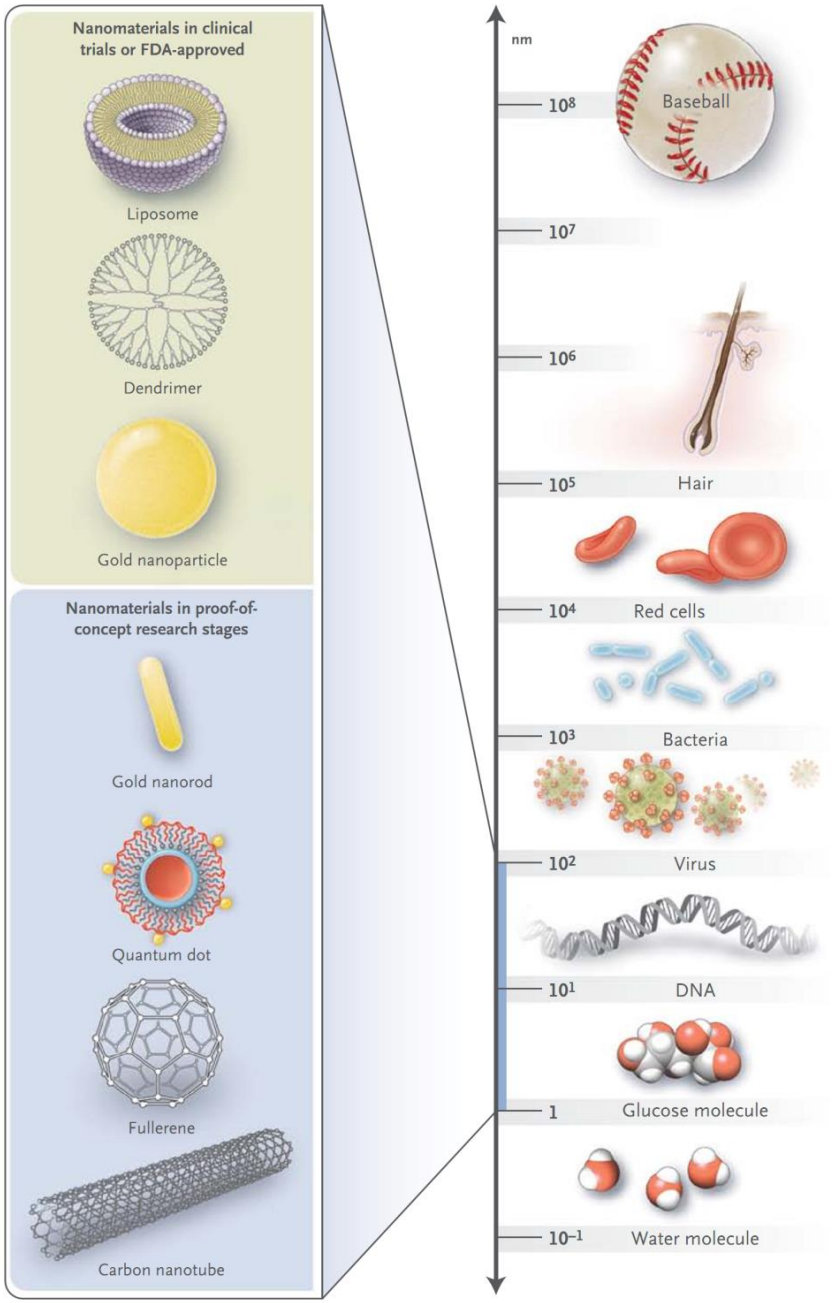
Nanoscale: size and range from approximately 1 nm to 100 nm

Nano-object: material with one, two or three external dimensions in the nanoscale



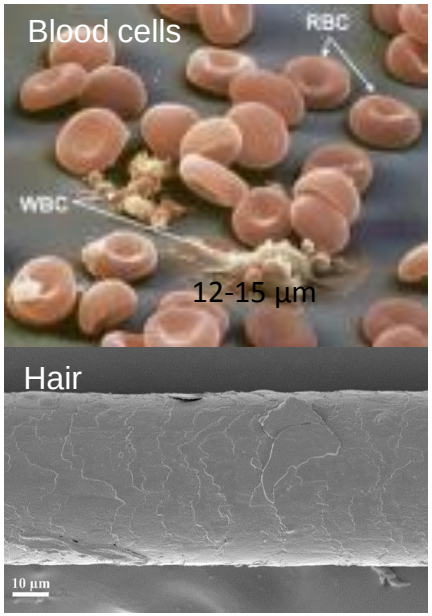
Regulation (EC) No 1223/2009:

Nanomaterial means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm

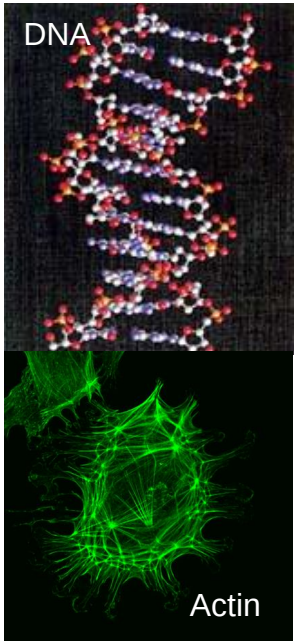


Betty *et al. 2010,* N Engl J Med 363:2534-43

“micro”



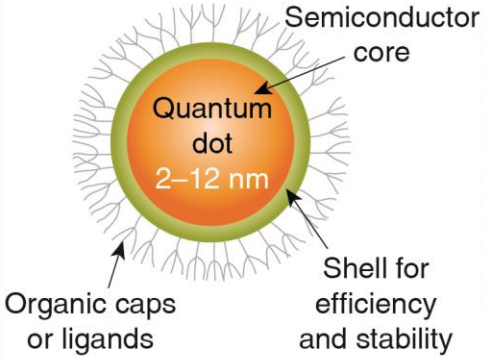
“nano”

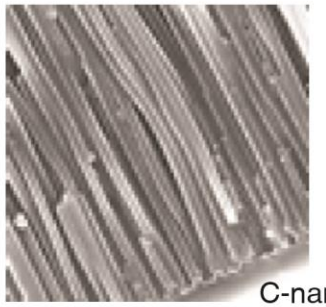


Nanomaterial classification by geometry and chemistry

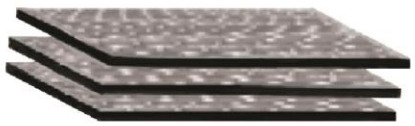
Chemistry \ Geometry	Isometric particles	1D; fibers/tubes	2D; plates, disks
Metals	Silver, gold nanoparticles Iron, cobalt, nickel magnetic NPs copper NP conducting inks	Gold or platinum nanowires	Silver nanoplates
Semiconductors	CdSe/ZnS quantum dots (see example)	Si, ZnO semiconducting nanowires, nanorods	Plate-like semiconductor nanocrystals
Ceramics	Zinc oxide, titanium dioxide pigments and sun screens, cerium oxide catalysts	Electrospun ceramic nanofibers for composite fillers	Nanoclays
Carbons	Fullerenes, carbon black, carbon nanohorns	Carbon nanotubes Carbon nanofibers	Graphene, graphene oxide few-layer graphene (example)
Polymers	Biodegradable polymer nanobeads for medical applications, branched dendrimers	Electrospun polymer nanofibers	

Examples:





C-nanofiber array

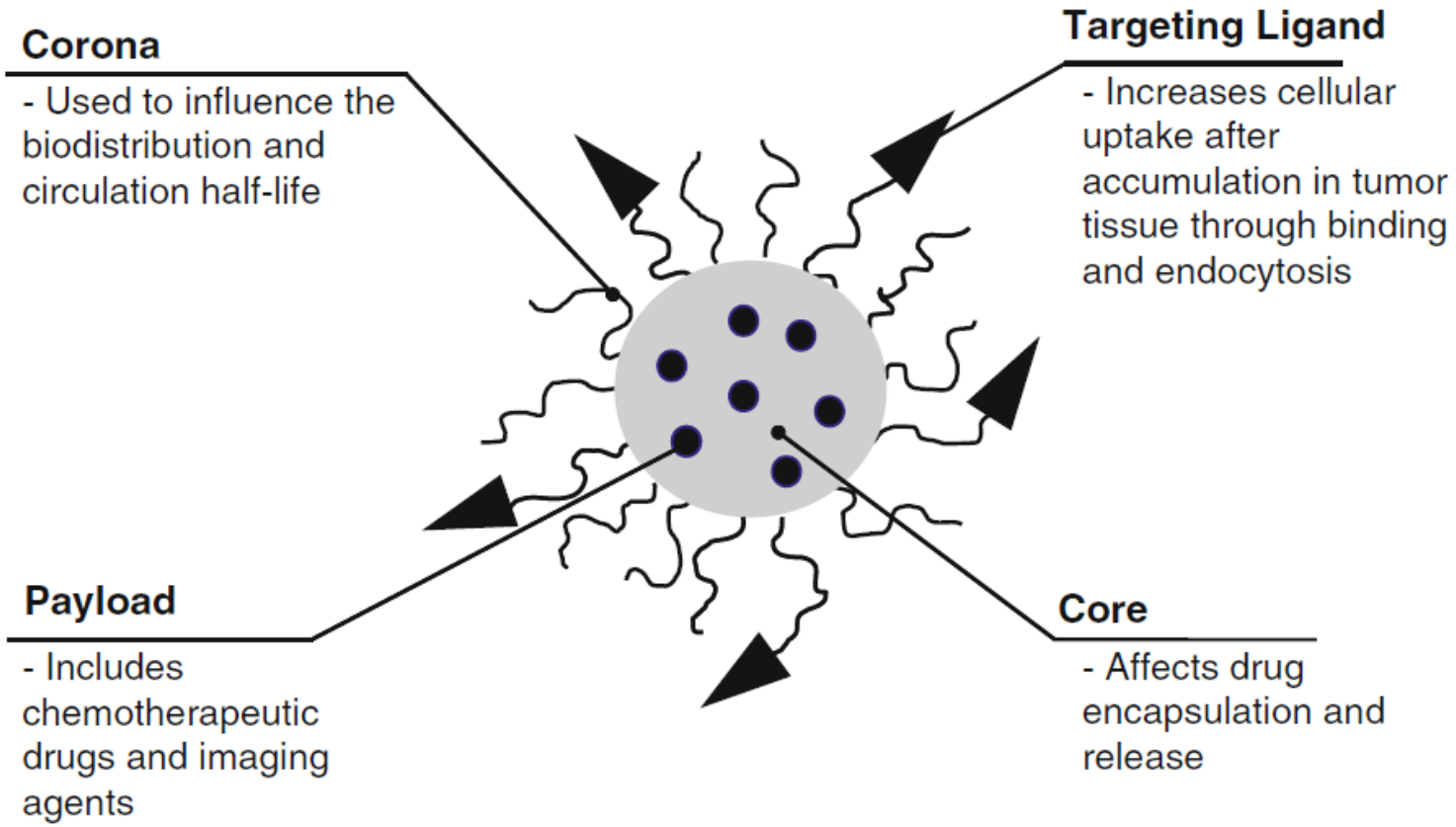


Few-layer graphene

Casarett & Doull's Toxicology, 8th Ed.

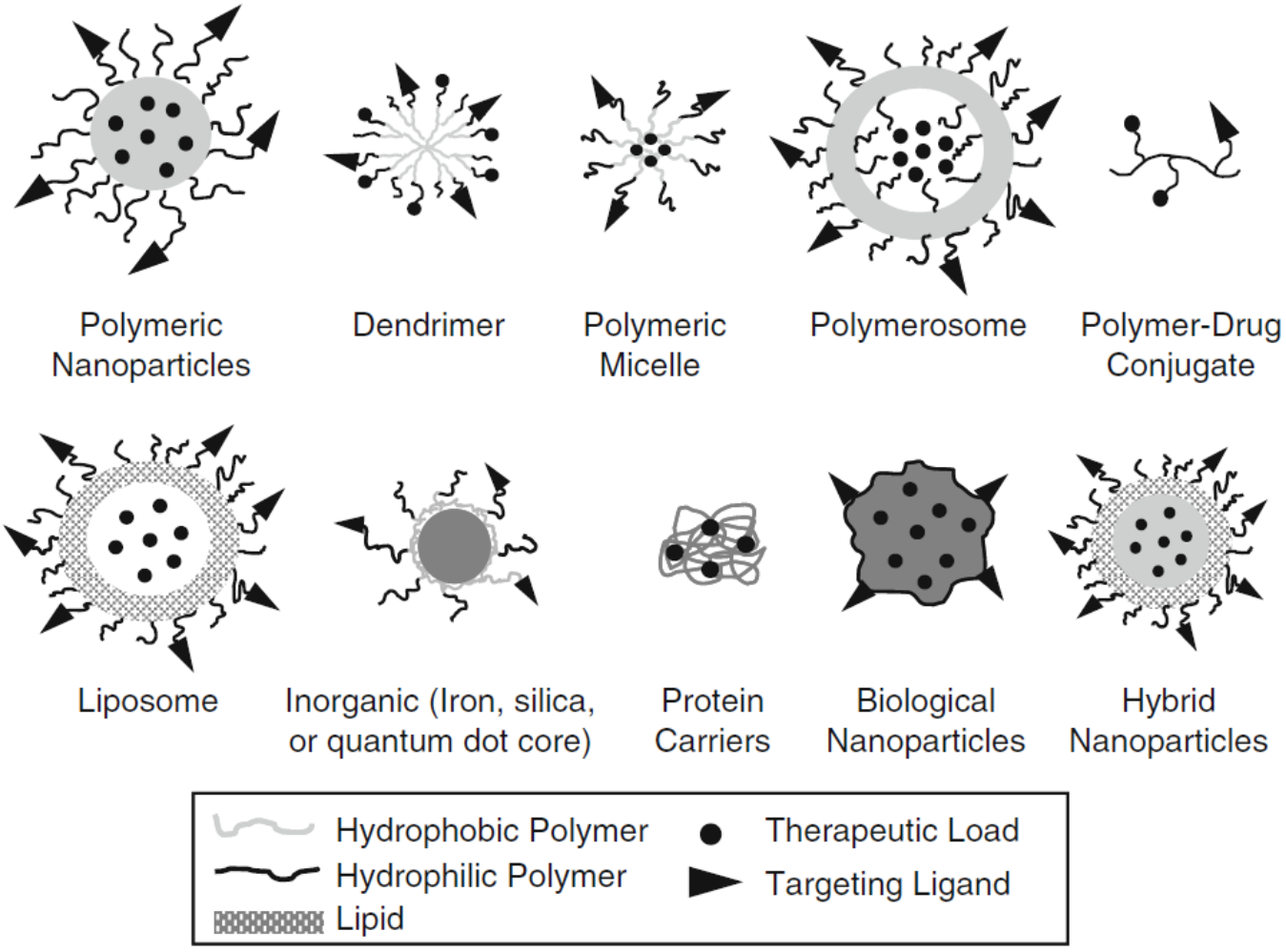
Nanoparticles for drug delivery

Physicochemical structure of Nanoparticle for drug delivery



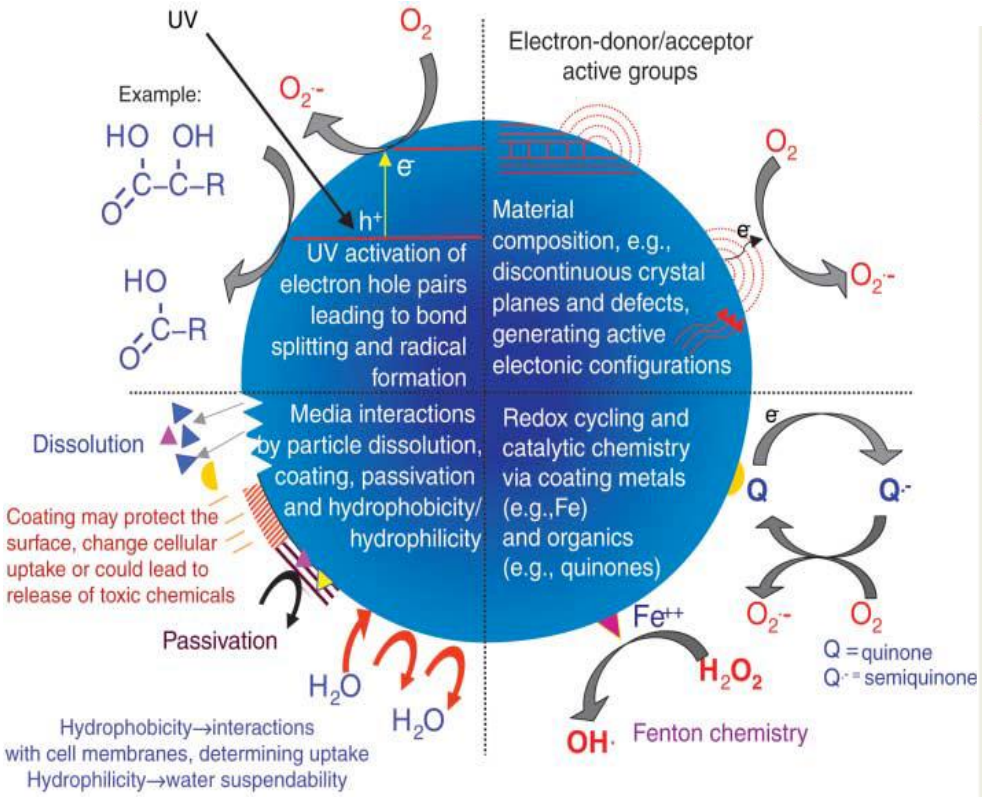
M. Schäfer-Korting (ed.), Drug Delivery, Handbook of Experimental Pharmacology 197, 2010.

Nanoparticle platforms for drug delivery



Physicochemical characteristics of nanomaterials

"Not only small, but different"



Nel et al., Science (2006) 311: 622-627

Physicochemical NP Properties Relevant to Toxicology

Size *(aerodynamic, hydrodynamic)*
Size distribution
Shape
Agglomeration/aggregation state
Density *(material, bulk)*
Chemical composition and phase
- crystallinity
- dissolution and toxicant (ion) release
- coatings and bioavailable contaminants
- biopersistence

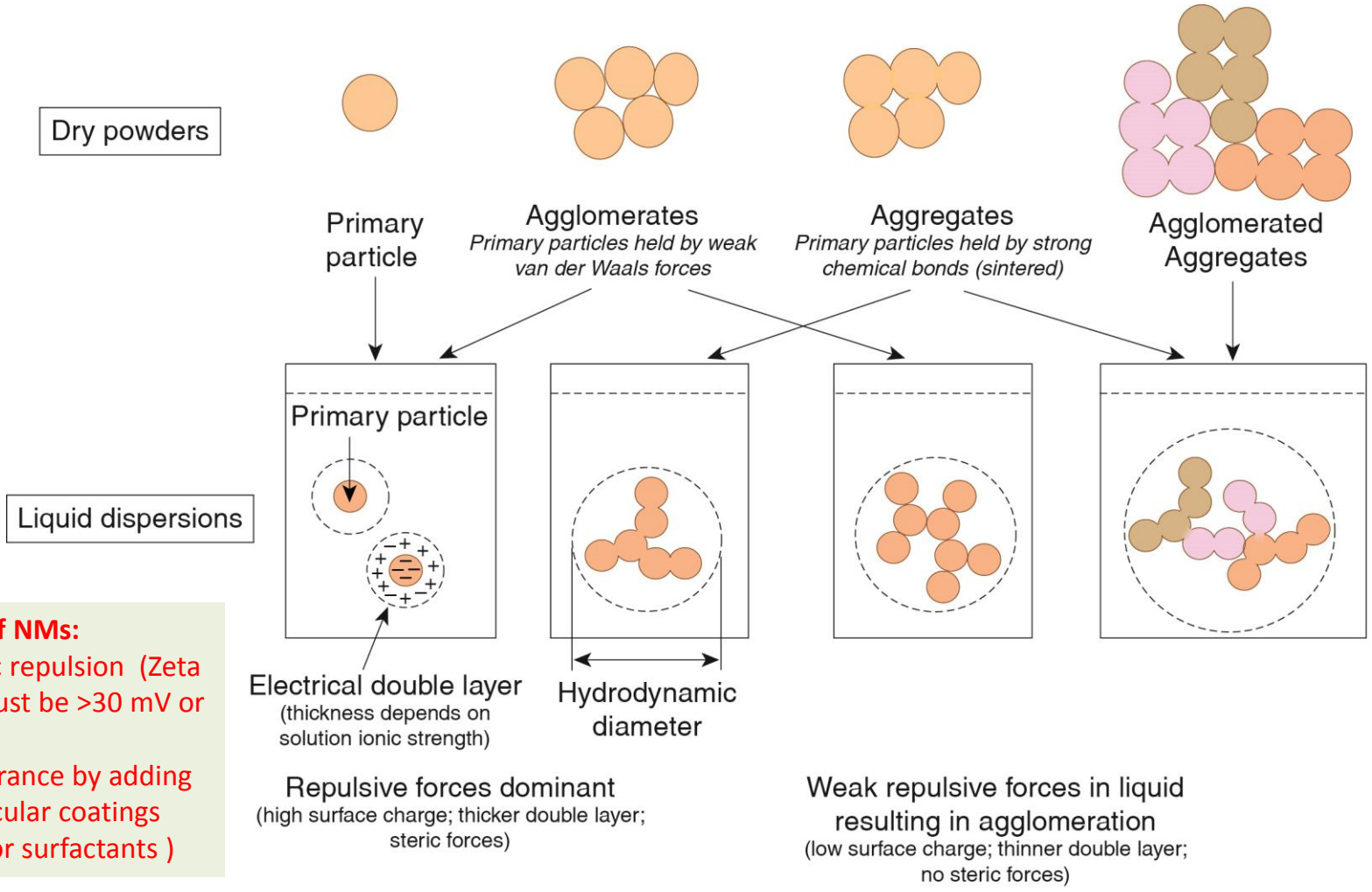
Surface properties
- surface area (external, internal)
- electrical charge (zeta potential)
- redox activity
- hydrophobicity/hydrophilicity
- adsorptive capacity for biomolecules

Nanoscale quantum and magnetic properties (?)

Properties can change
- with method of production, preparation process, storage
- when introduced into physiol. media, organism

Casarett & Doull's Toxicology, 8th Ed.

Agglomeration and aggregation of nanoparticles

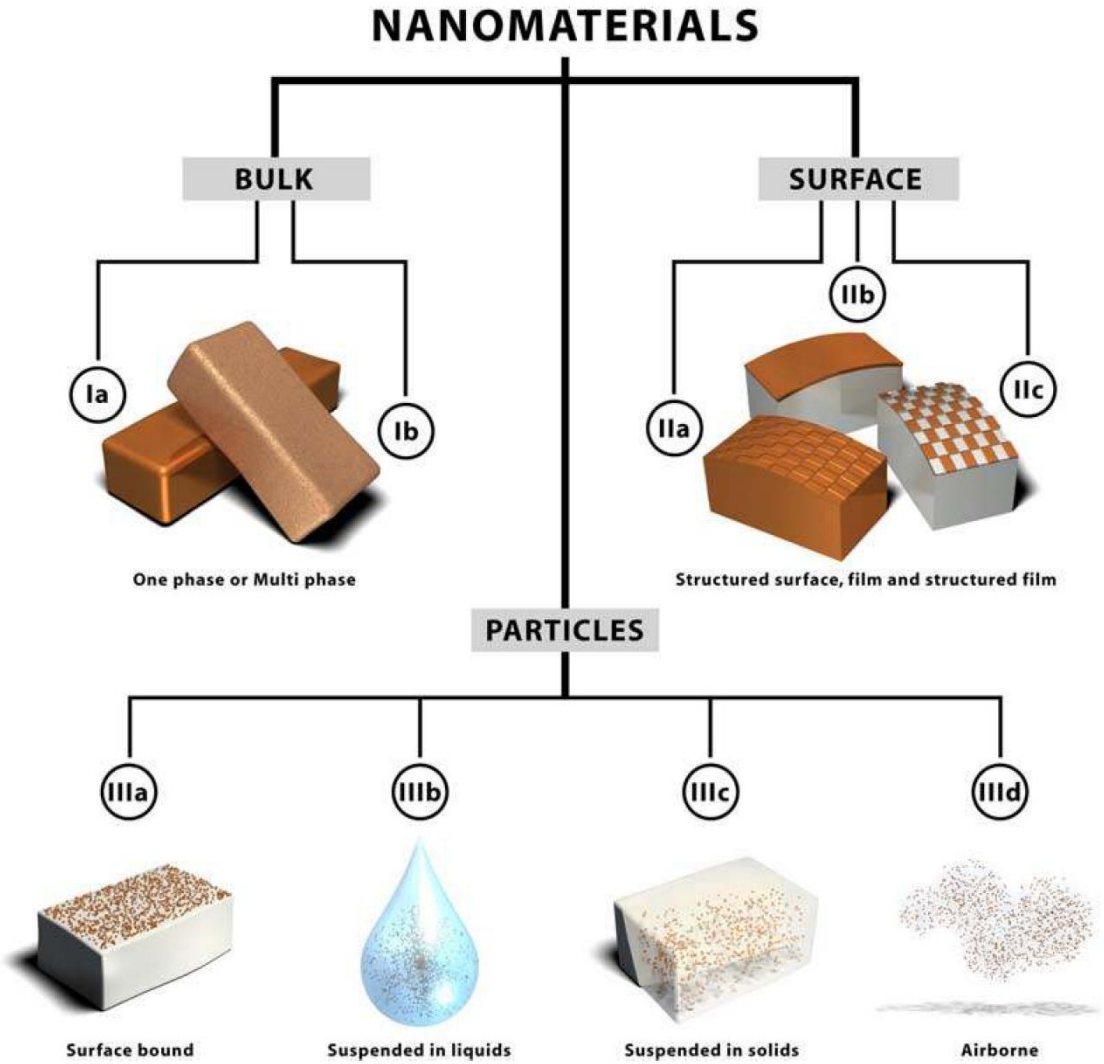


Stabilization of NMs:

1) Electrostatic repulsion (Zeta potential must be >30 mV or <-30 mV)
2) Stearic hindrance by adding macromolecular coatings (polymers or surfactants)

Casarett & Doull's Toxicology, 8th Ed.

Categorization framework for nanomaterials

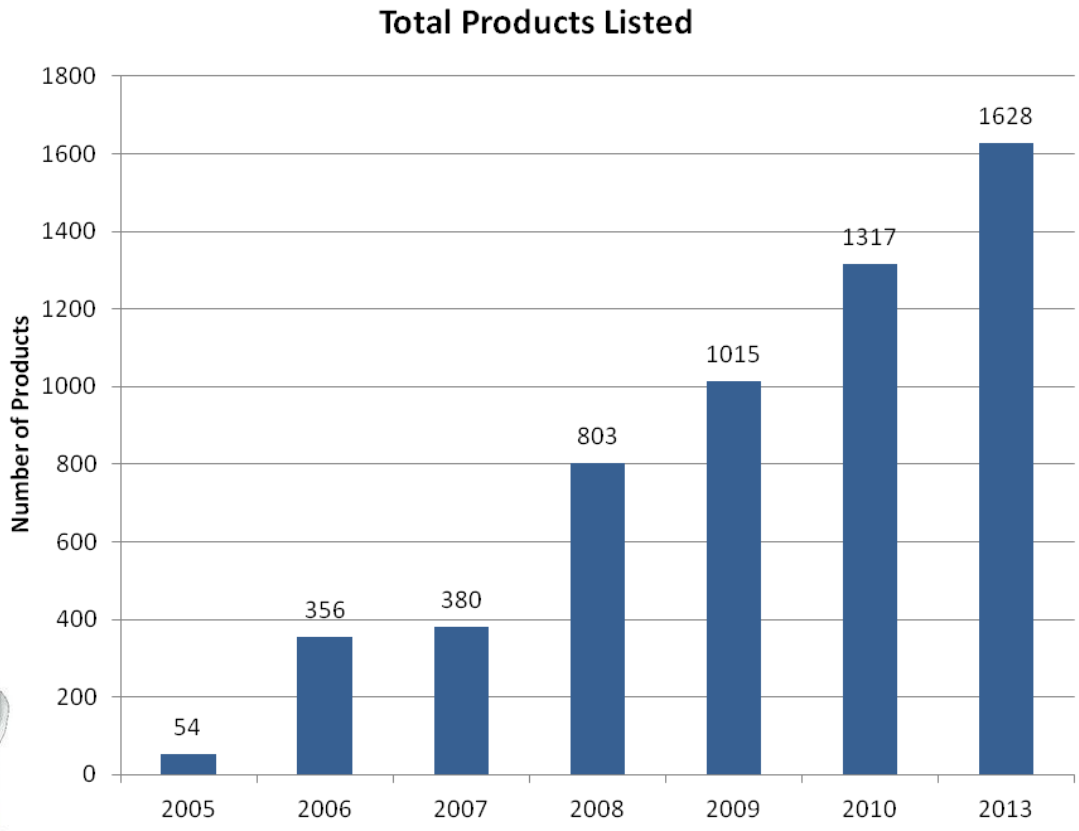


The nanomaterials are categorized according to the location of the nanostructure in the materials

Hansen et al., *Nanotoxicology* 2007

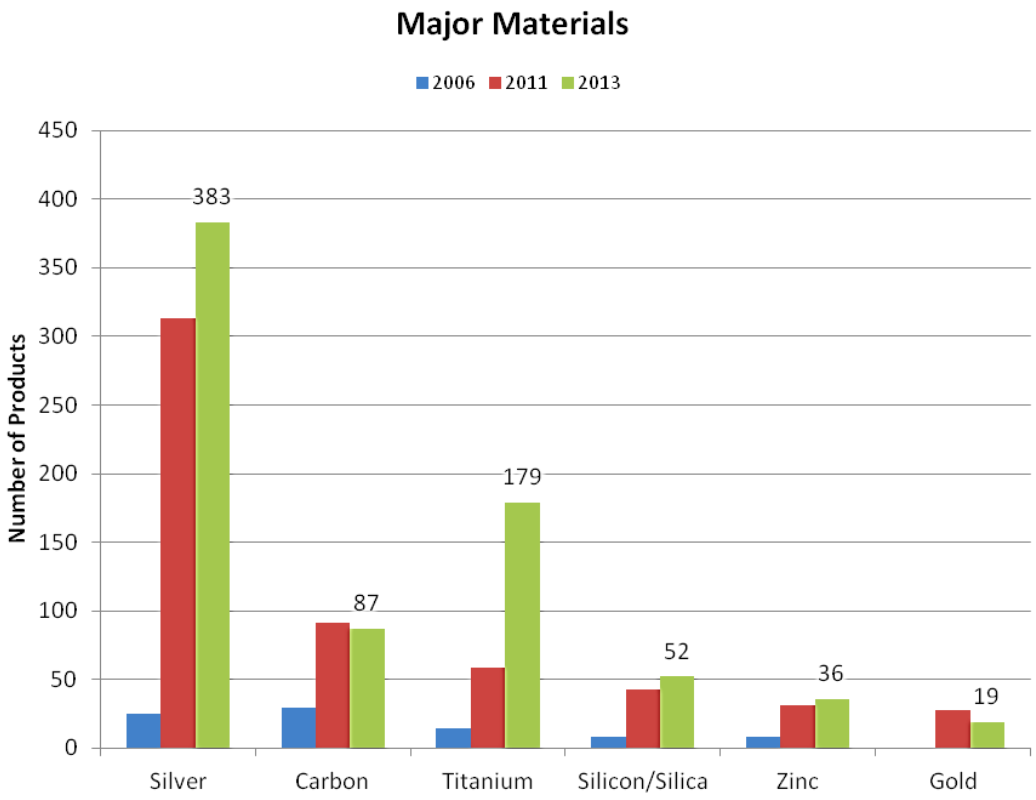
Nanoproducts: from past to present

- Textiles
- Cosmetics
- Medical devices
- Food supplements
- Personal care products
- House hold products



http://www.nanotechproject.org/cpi/about/analysis/

Nanomaterials Used in Commercial Products



http://www.nanotechproject.org/cpi/about/analysis/

Selected nanomaterial markets

Selected nanomaterials market	Global market revenue by 2016, USD billion	Expected global market revenue by 2021, USD billion	Expected CAGR in 2016-2021, percent
Silver nanoparticles	1.1	3.0	13
Nanoclays	0,7	2,1	24,9
Nanocomposites	1.6	5.3	26.7
Quantum dots	0.61	3.4	41.3
Nanofibers	0.39	2.0	38.6
Advanced & nanoscale ceramic powders	14.6	22.3	8.9

Source: Silver Nanoparticles Market Size (2017); Global Silver Nanoparticles Market Trends (2017); Nanocellulose Market (2015); Nanoclay market (2016)

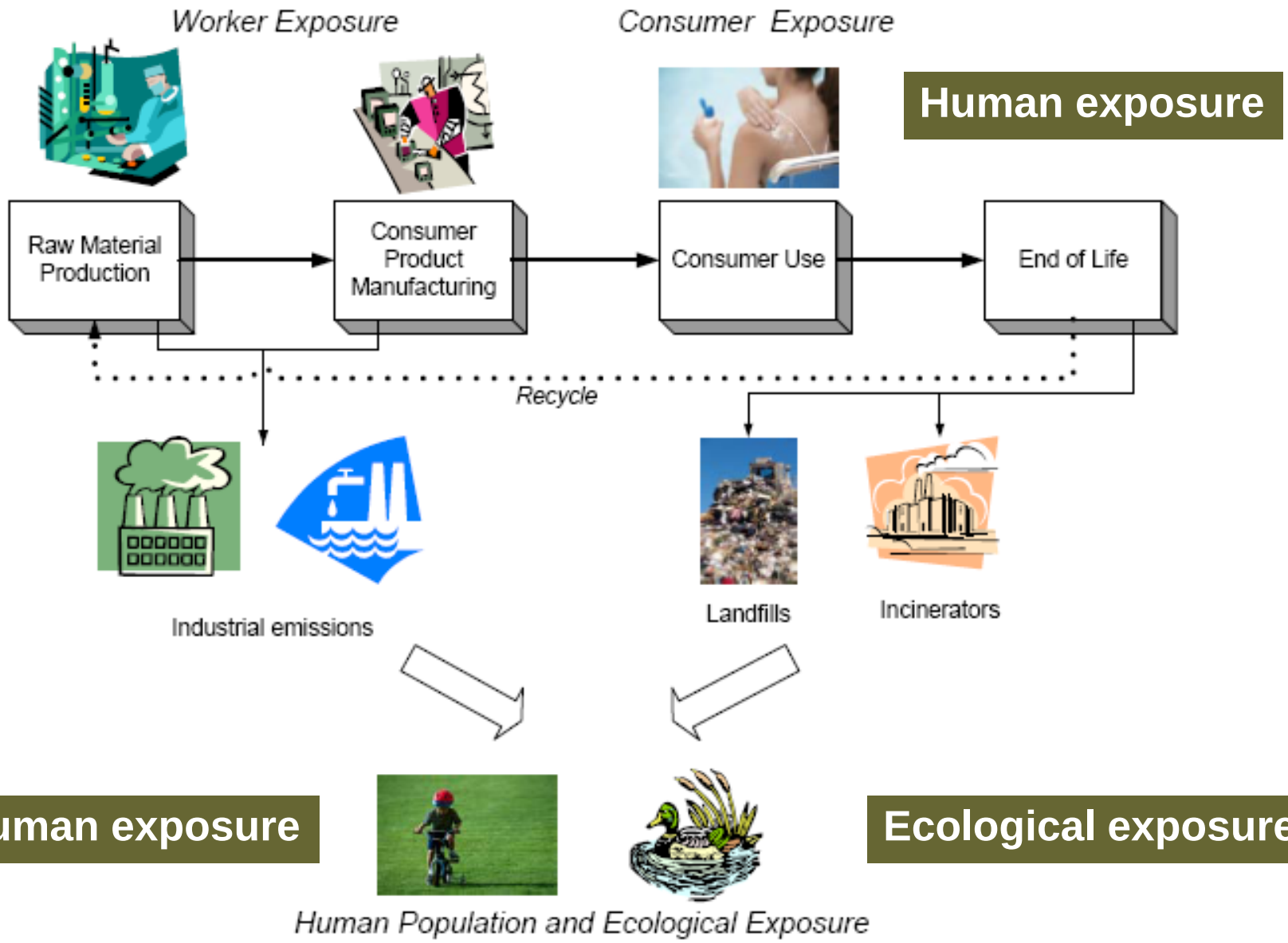
Production quantities of NMs (ton/year) for Switzerland

	Mode	Mean	SD
nano-TiO_2	114	240	195
CNT[b]	1.9	2.6	1.2
nano-Ag	1.1	2.3	1.7

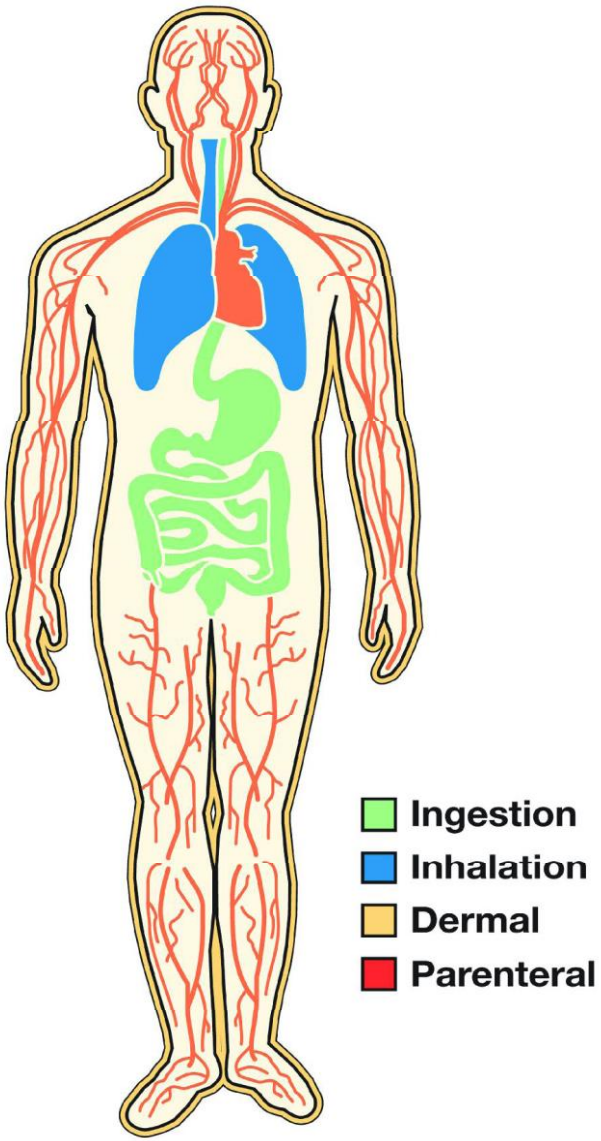
Gottschalle et al. (2010) , Environ Toxicol Chem. 29:1036-48.

Exposure to Nanomaterials

Nanotechnology Life Cycle Perspective



Potential routes of human exposure



Inhalation: Lung

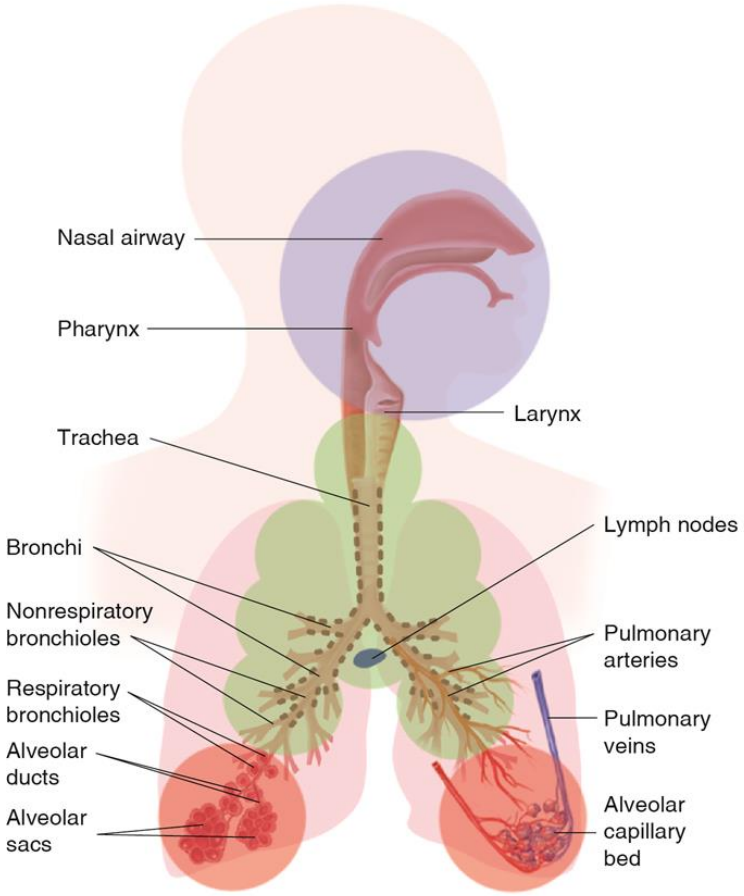
Ingestion: Gastro-intestinal tract

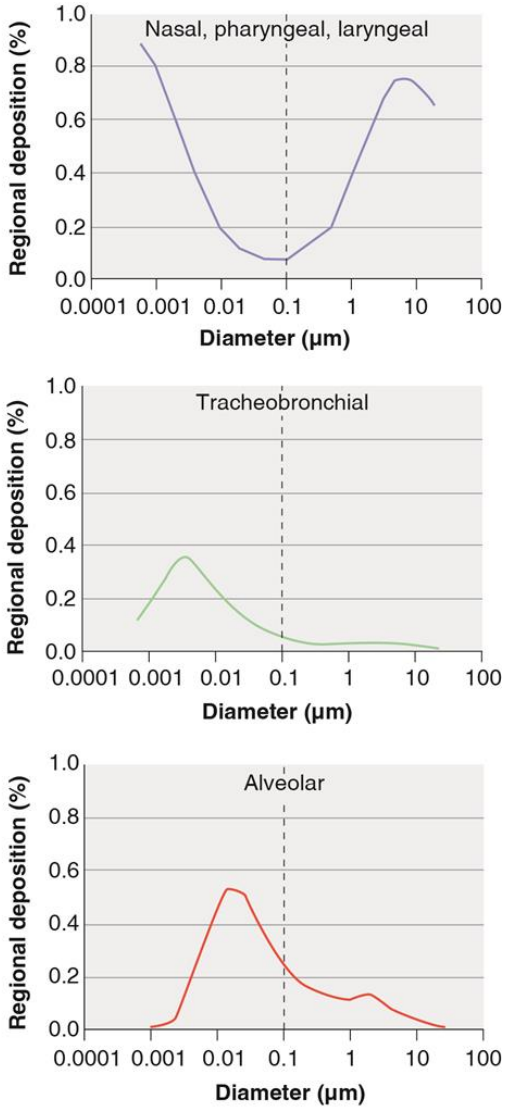
Dermal: Skin

Parenteral: Blood circulation

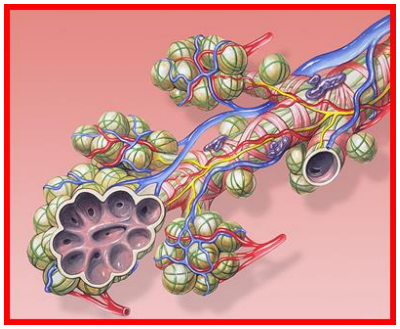
Local / Systemic adverse effects

Inhalation Exposure



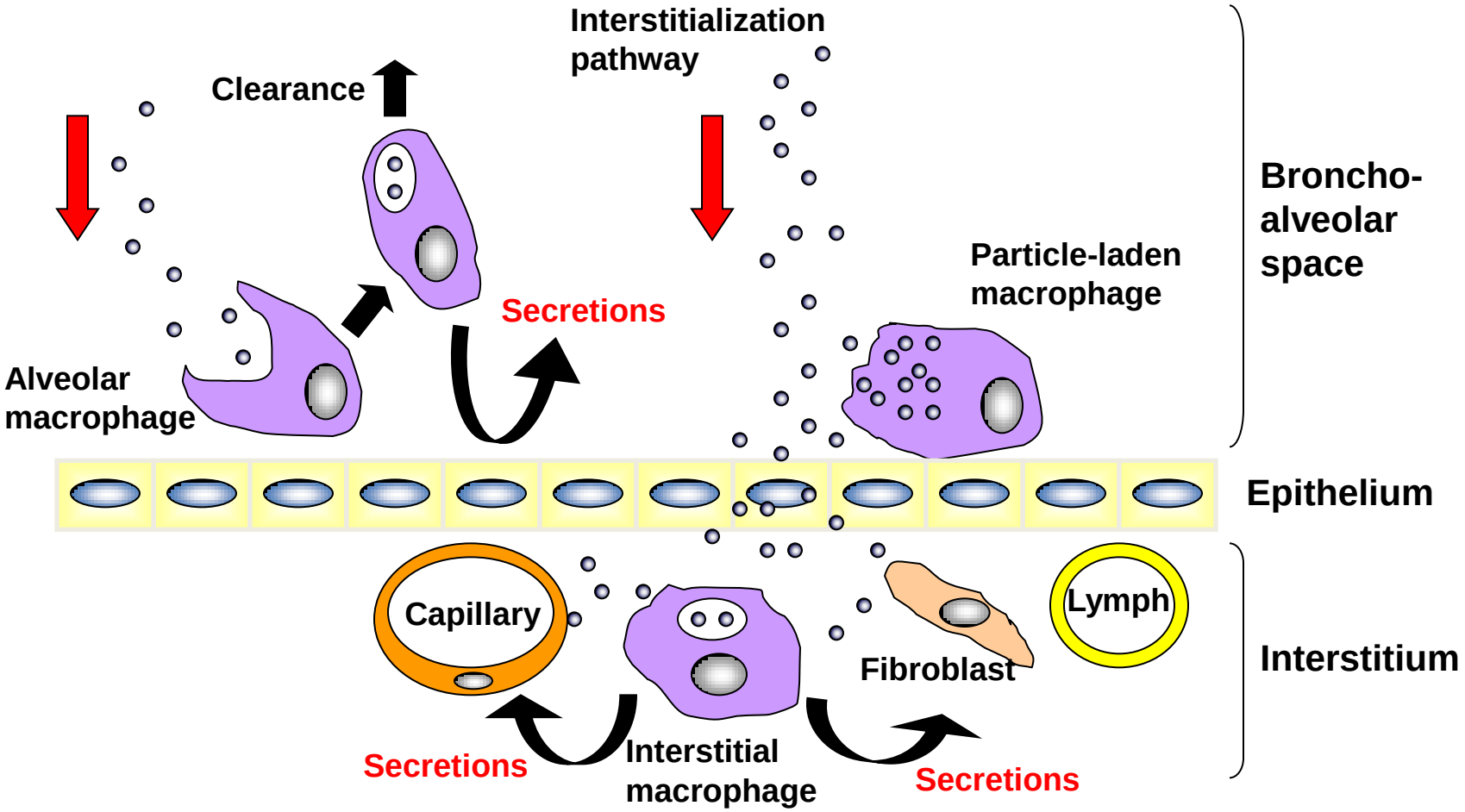


Alveoli



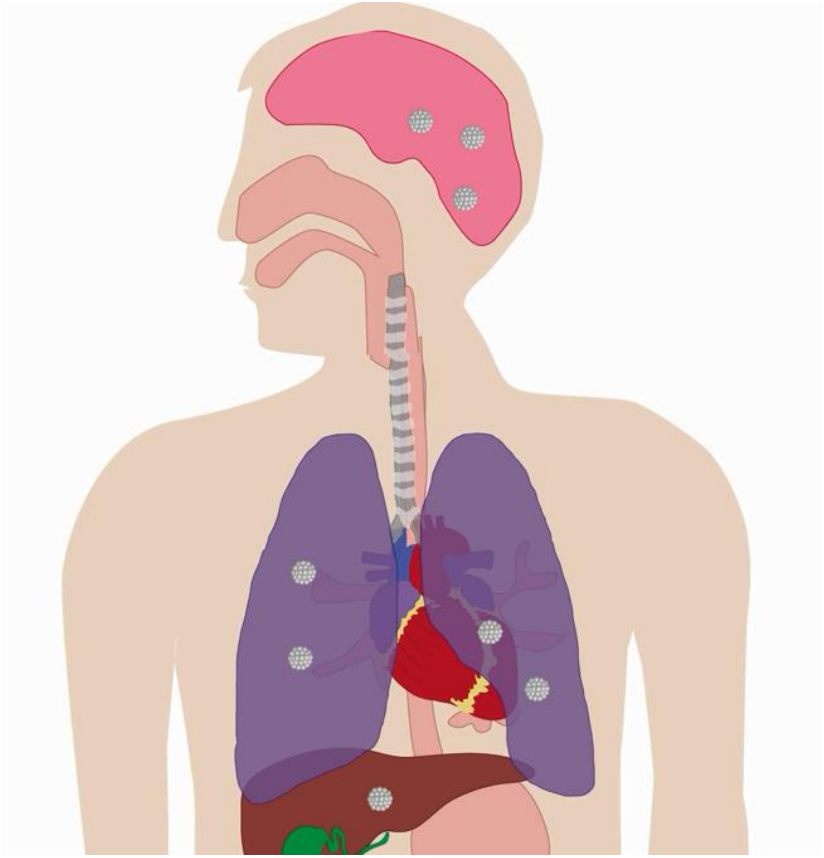
Oberdorster et al. (2005) *Environ Health Perspect*, 113:823–839.

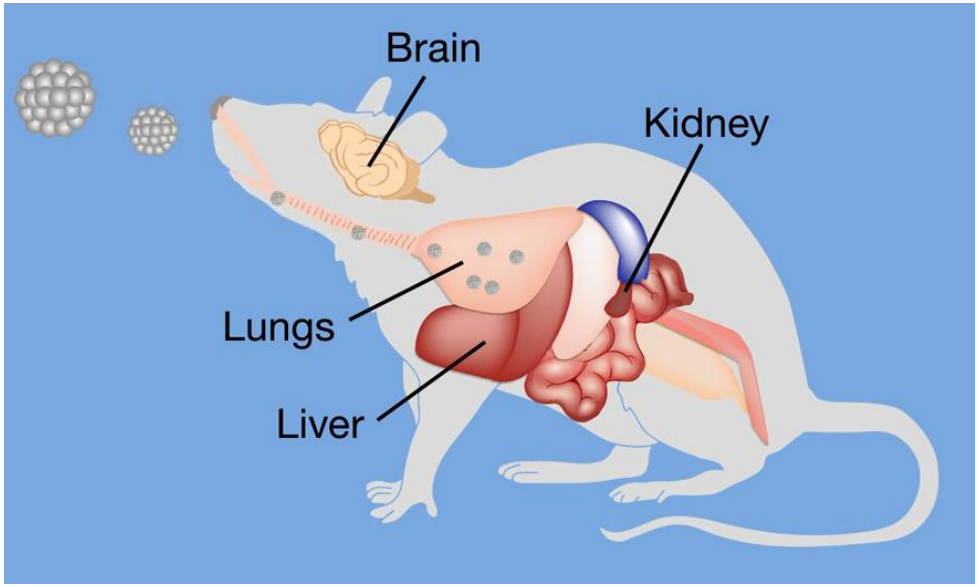
Potential Pathway for Nanoparticles in the Lung



Modified from Donaldson et al. 1998, J Aerosol Sci, 29: 553-560.

Translocation of NPs via inhalation

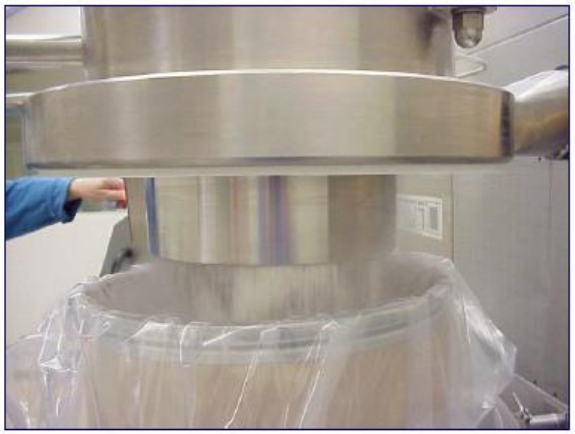




Video abstract summarizing new research published in Particle and Fibre Toxicology. "Slow lung clearance and limited translocation of four sizes of inhaled iridium nanoparticles" by Rachel Smith et. al 2017.

Inhalation exposure

The most common route of exposure to any aerosol particle in the workspace



wikipedia.org

Source: Nano safety course, Malaysia, Nov, 2018

https://www.cdc.gov/niosh/topics/aerosols/default.html

Inhalation exposure

What is Different: Nanoparticles Versus Larger Particles (*respiratory tract as portal-of-entry*)

	NANOPARTICLES (<100 nm)	LARGER PARTICLES (>500 nm)
General characteristics:		
Ratio: number or surface area/volume or mass	high	low
Agglomeration in air, liquids	likely (dependent on medium and surface)	less likely
Deposition mechanism in respiratory tract	diffusion; throughout resp. tract	sedimentation, impaction, interception; throughout resp. tract
Protein/lipid adsorption in vitro	very effective and important	less effective
Protein/lipid adsorption in vivo	yes	some
Translocation to secondary target organs:	yes	generally not (to liver under "overload")
Clearance		
• mucociliary	probably yes	efficient
• by alveolar macrophages	poor	efficient
• into or across lung epithelium	yes	mainly under overload
• lymphatic	yes	under overload
• blood circulation	yes	under overload
• sensory neurons (uptake + transport)	yes	not likely
Cell entry/uptake	yes (caveolae; clathrin; lipid rafts; diffusion)	yes (primarily phagocytic cells)
• mitochondria	yes	no
• nucleus	yes (<40 nm)	no
Effects (*caveat: dose!*):		
at secondary target organs	yes	(no)
at portal of entry (resp. tract)	yes	yes
• inflammation	yes	yes
• oxidative stress	yes	yes
• activation of signaling pathways	yes	yes
• genotoxicity, carcinogenicity	probably yes	some

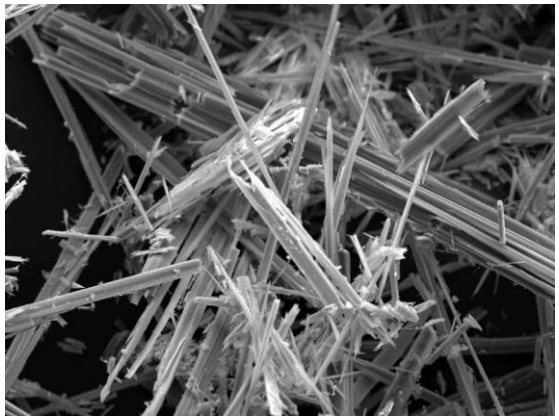
Data from: Oberdörster et al., 2009

Casarett & Doull's Toxicology, 8th Ed.

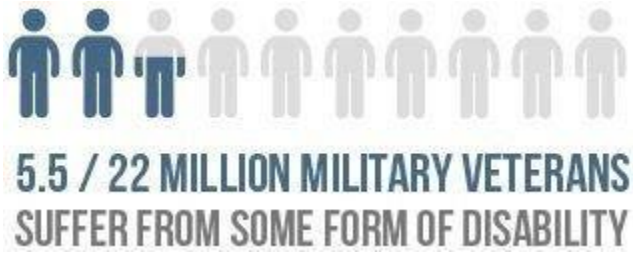
Hazards of fibrous particles: asbestos and lung disease

Asbestos

- Minerals with long, thin fibers found naturally in rocks and soil.
- Used in a wide range of household, commercial, and industrial products
- When these fibers are inhaled or ingested, they can cause serious asbestos-related diseases such as mesothelioma, lung cancer, and asbestosis.

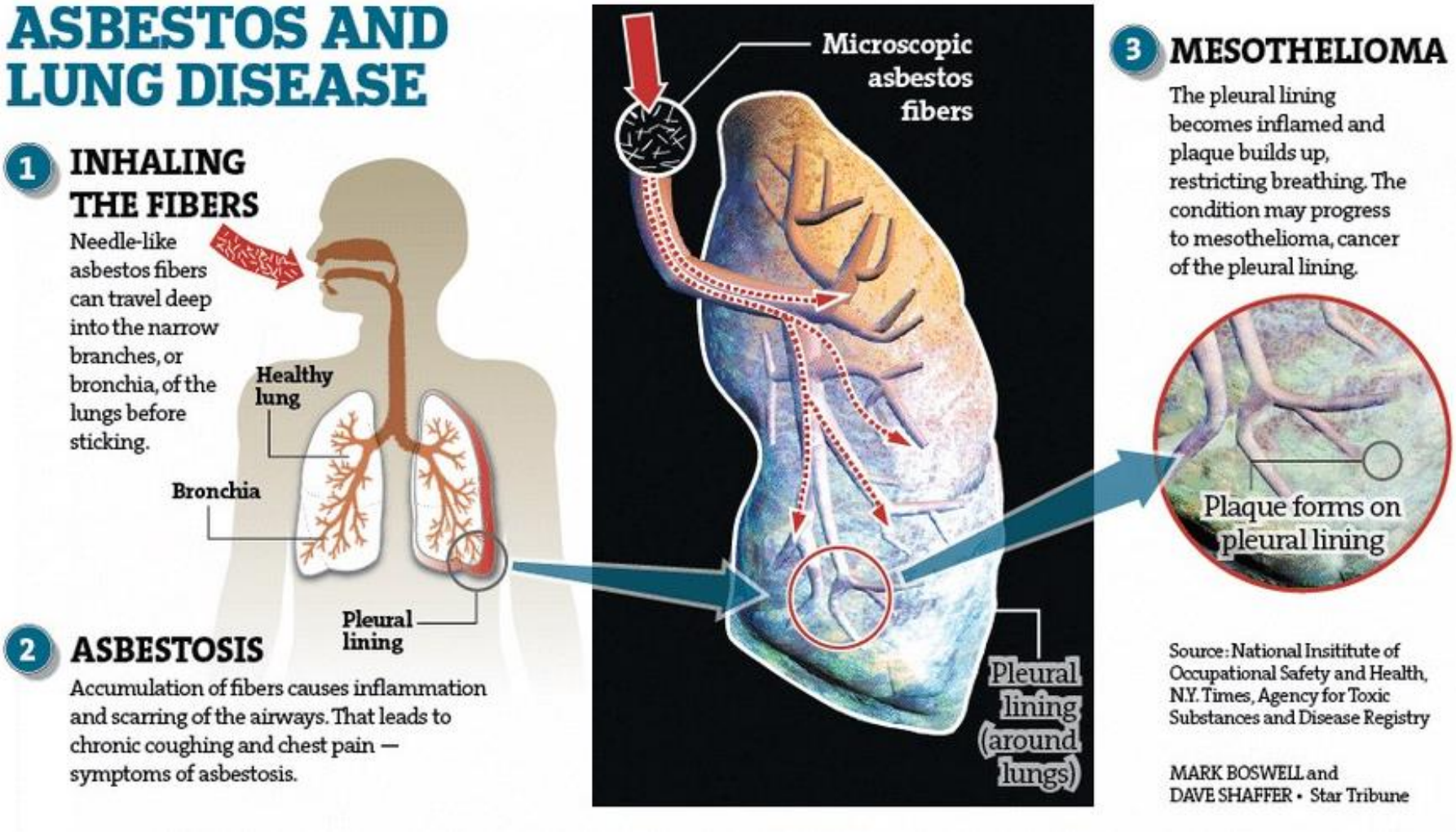


https://www.smithsonianmag.com



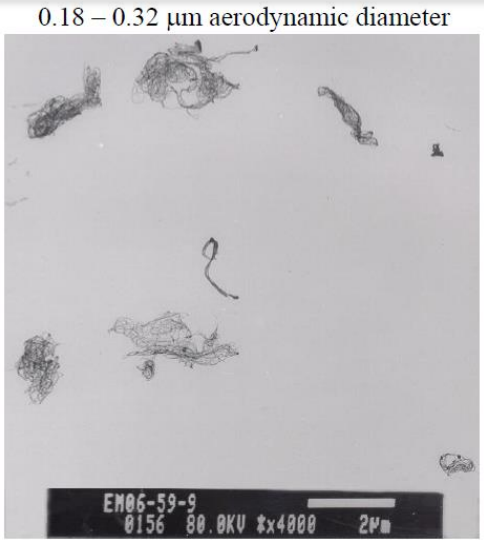
Despite its proven health hazards as a carcinogen, asbestos is still not banned in the United States. And for many years, asbestos companies knew of its danger but failed to warn people.

https://www.mesotheliomahelp.org/asbestos/

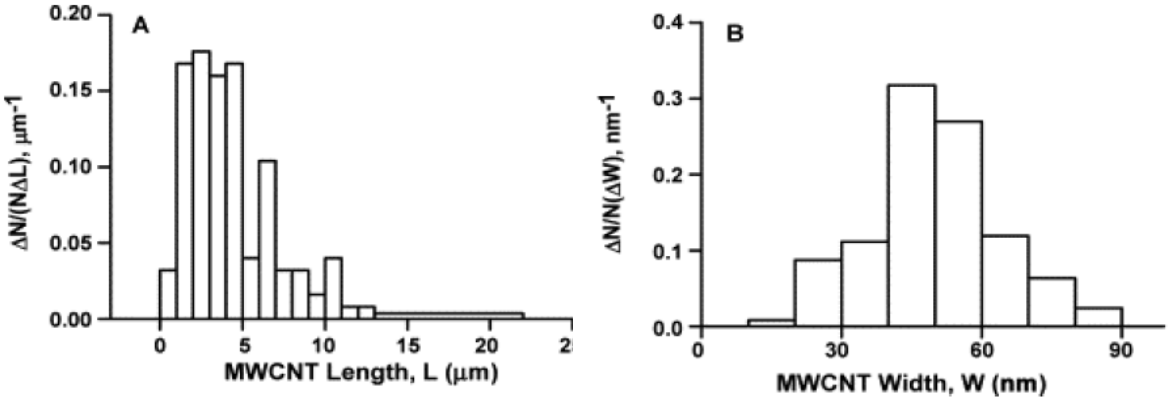

ASBESTOS AND LUNG DISEASE
1 INHALING THE FIBERS
Needle-like asbestos fibers can travel deep into the narrow branches, or bronchia, of the lungs before sticking.
Healthy lung
Bronchia
Pleural lining
2 ASBESTOSIS
Accumulation of fibers causes inflammation and scarring of the airways. That leads to chronic coughing and chest pain — symptoms of asbestosis.
Microscopic asbestos fibers
Pleural lining (around lungs)
3 MESOTHELIOMA
The pleural lining becomes inflamed and plaque builds up, restricting breathing. The condition may progress to mesothelioma, cancer of the pleural lining.
Plaque forms on pleural lining
Source: National Insititute of Occupational Safety and Health, N.Y. Times, Agency for Toxic Substances and Disease Registry
MARK BOSWELL and DAVE SHAFFER • Star Tribune

Fibrous NMs: CNTs

NPs have a tendency to agglomerate and form larger structures, which influences their dynamics in the environment and deposition on biological surfaces.



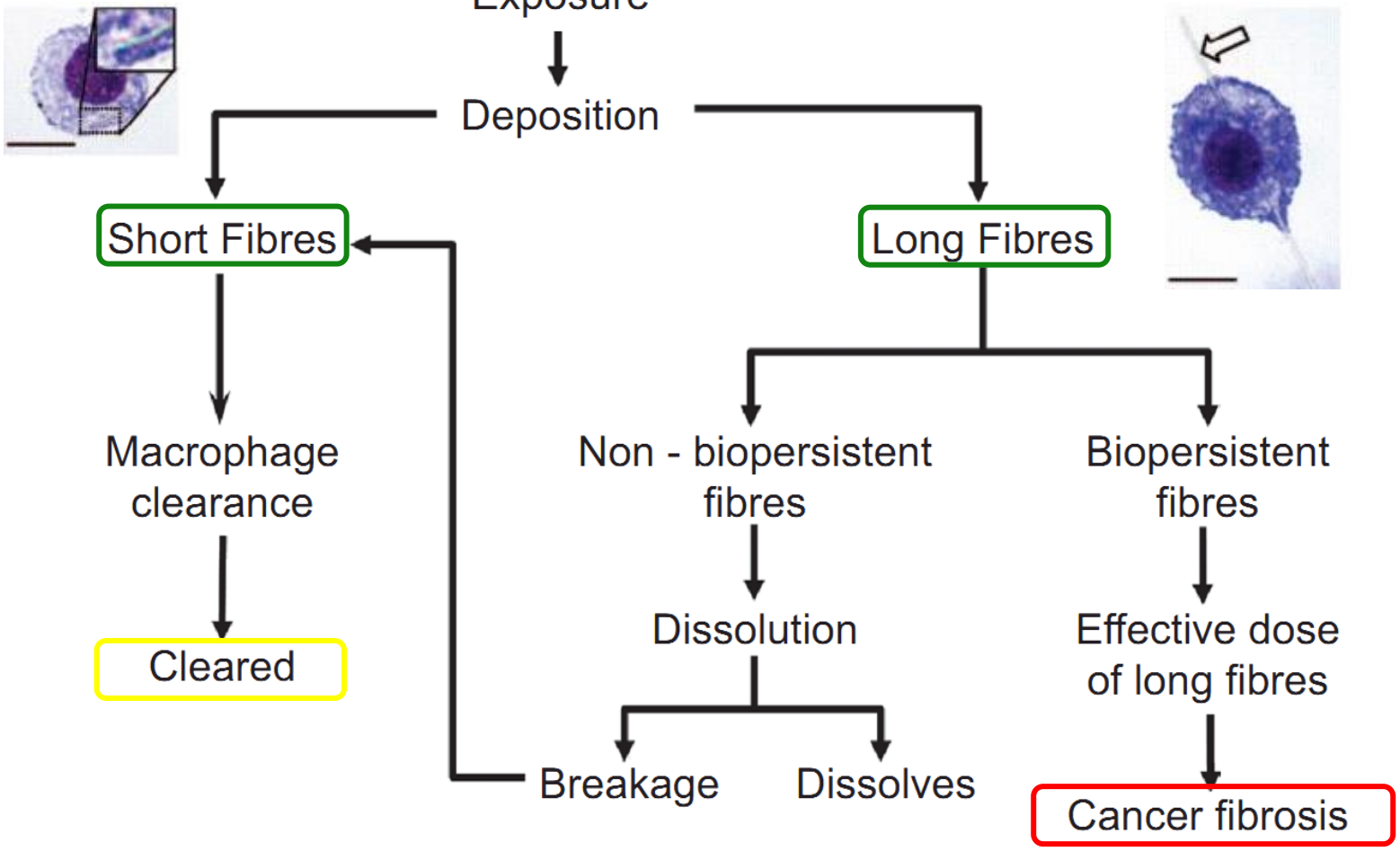
Commercial samples of MWCNT contain mixtures of MWCNT with varying width and length



The length of CNT fibers is critical in determining whether they would result in asbestos-like pathogenicity.

Source: Nano safety course, Malaysia, Nov, 2018

Overarching model for the role of length, biopersistence, and clearance in the fiber pathogenicity paradigm



Adapted from: Donaldson K., Critical Reviews in Toxicology, 2009; 39: 487-500.

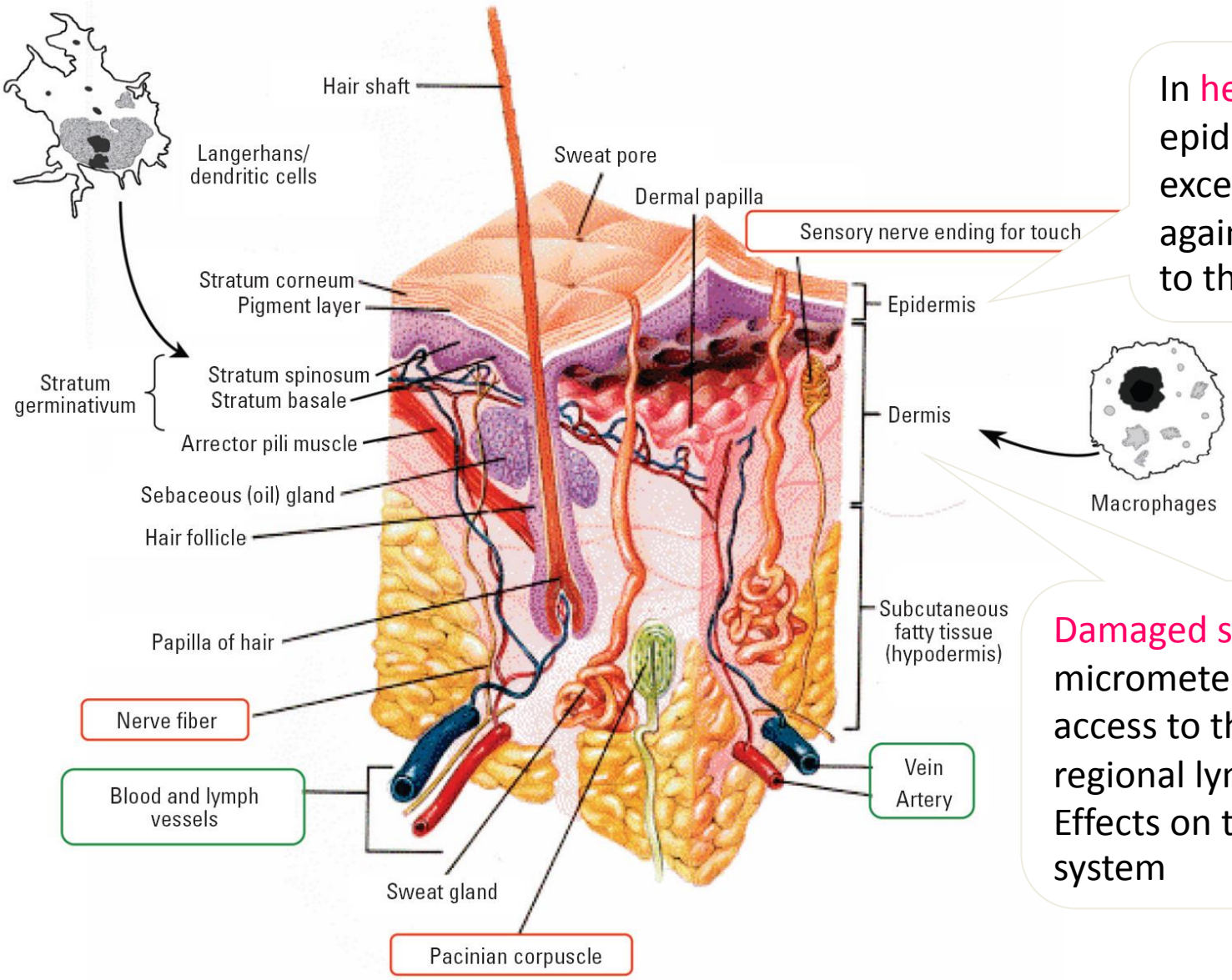
Inhaled particles

Parameters that play a major role in determining toxic response upon inhalation: **"D words"**

- **D**ose : Cumulative dose for chronic effects (particle/fiber mass or number or surface dose)
- **D**imension : Size (diameter and length)
- **D**eposition : Depend on dimension but also on airway properties
- **D**urability : Biopersistence dependent on defense and particle properties (dissolution)
- **D**efense : Mucociliary clearance, macrophage clearance, inflammatory cells

Borm et al., Int J Cancer, 2004: 3-14.

Dermal Exposure



In healthy skin, the epidermis provides excellent protection against particle spread to the dermis

Damaged skin allows micrometer-size particles access to the dermis and regional lymph nodes → Effects on the immune system

Penetration through skin barrier

- The stratum corneum is an excellent skin barrier
- Factors influence in penetration test for nanomaterials
  - Hair follicle density
  - Size of hair follicle opening
  - Lipid structures and contents

Species difference in hair follicle density

Species	Area	Number of hair follicles/cm^2
Human	Abdomen	11 ± 1
Pig	Back	11 ± 1
Rat	Back	289 ± 21
Mouse	Back	658 ± 38
Hairless mouse	Back	75 ± 6

Bronaugh et al. 1982, Toxicol Appl Pharmacol 62: 481-488.

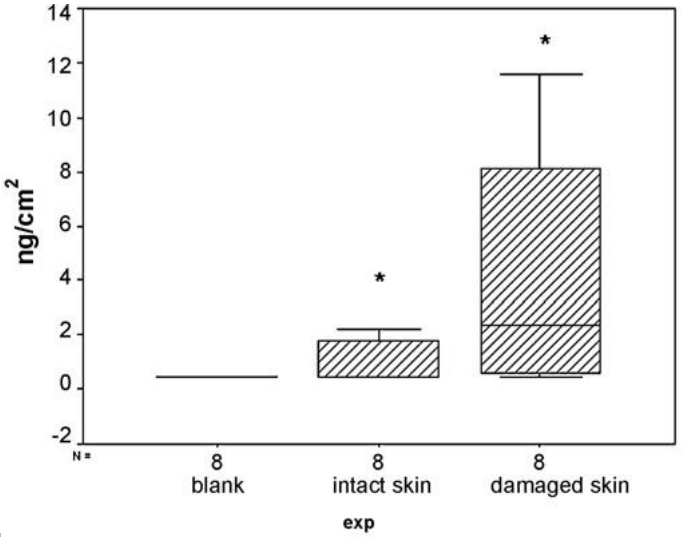
- The skin from furry rodents results in overestimation of human skin penetration
- Pig has different lipid structures from human

Human skin penetration of silver nanoparticles through intact and damaged skin

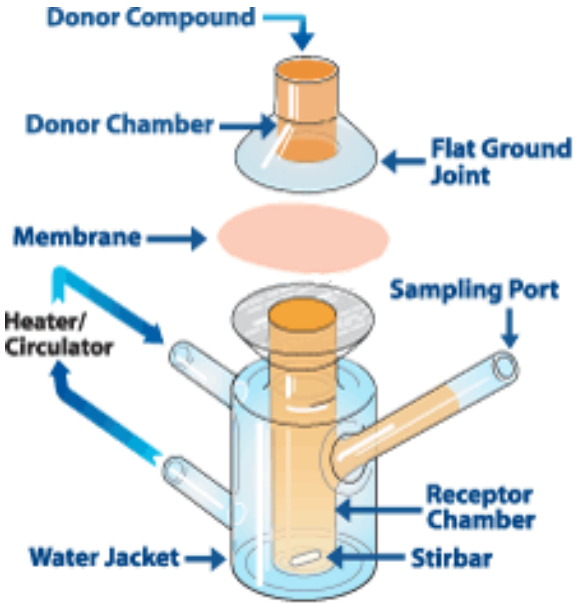
Larese et al. (2009), *Toxicology* 255: 33-37.

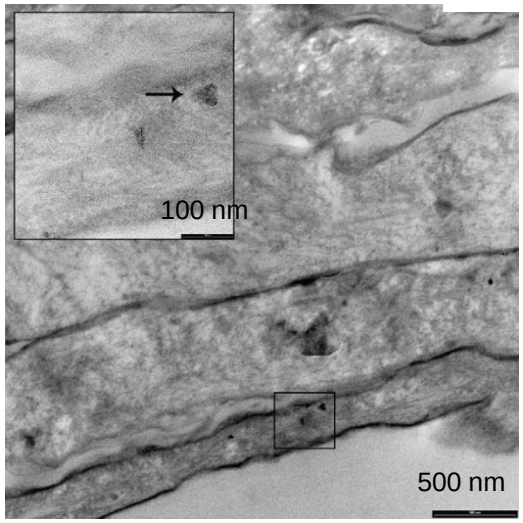
Human abdominal full thickness skins

Silver nanoparticles (25±7.1 nm)



Franz diffusion cell method





Silver skin penetration at 24 h

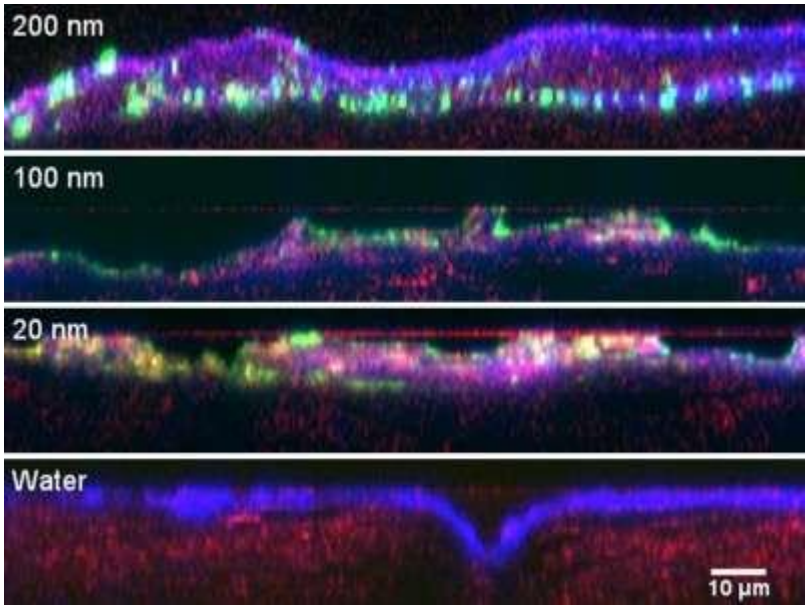
TEM micrograph of Ag nanoparticles-treated skin sample

Ag nanoparticles are presented in **deep stratum corneum**

Dermal exposure

"Do NPs penetrate the skin?"

- Several studies show little or no penetration of NPs beyond surface skin



Confocal laser scanning microscopy image of polystyrene beads (green) (20 - 200 nm) absorbed into the skin.
https://phys.org/news/2012-10-nanoparticles-dont-penetrate-skin.html

Study on penetration of TiO_2 NPs through the skin by Makiko Fujii

1-35 nm TiO_2 NPs with an alumina/silica coating showed no penetration to deeper skin layers.

Investigation of TiO_2 NPs penetration through skin in sunscreen formulations by P. Moretto

Diffusion of NPs was only limited to upper layers of the stratum corneum. No penetration through skin furrows or follicular openings in the skin was observed.

https://ninithi.wordpress.com/2015/08/17/nan-safety-update-scientists-invasively-tested-human-for-nanoparticle-penetration-they-were-in-for-a-surprise/

แนวทางการทดสอบผลิตภัณฑ์เครื่องสำอางที่มีวัสดุนาโน

Guidance on the Safety Assessment of Nanomaterials in Cosmetics (2012)


SCCS/1484/12

Scientific Committee on Consumer Safety

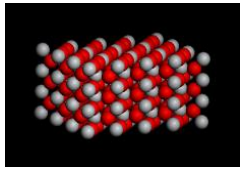
SCCS

GUIDANCE ON THE SAFETY ASSESSMENT OF NANOMATERIALS IN COSMETICS

The SCCS adopted this opinion at its 15th plenary meeting of 26 - 27 June 2012

Nanomaterial means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm

[Regulation (EC) No 1223/2009]



Cosmetic ingredients:

Physicochemical characterization (considering “nano” aspect)

- **Chemical identity**
- **Chemical composition**
- **Purity, impurities**
- **Size**
- **Morphology**
- **Surface characteristics**
- **Solubility**
- **Surface area**
- **Catalytic activity**

etc.

Main toxicological endpoints:

- Percutaneous absorption
- Toxicokinetics
- Acute toxicity
- Irritation and corrosiveness
- Skin sensitization
- Mutagenicity/ genotoxicity
- Repeated dose toxicity
- Carcinogenicity
- Reproductive toxicity
- Photo-induced toxicity
- Human data

etc.

Oral Exposure

The gastrointestinal tract represents a likely route of entry for many nanomaterials, both directly or indirectly

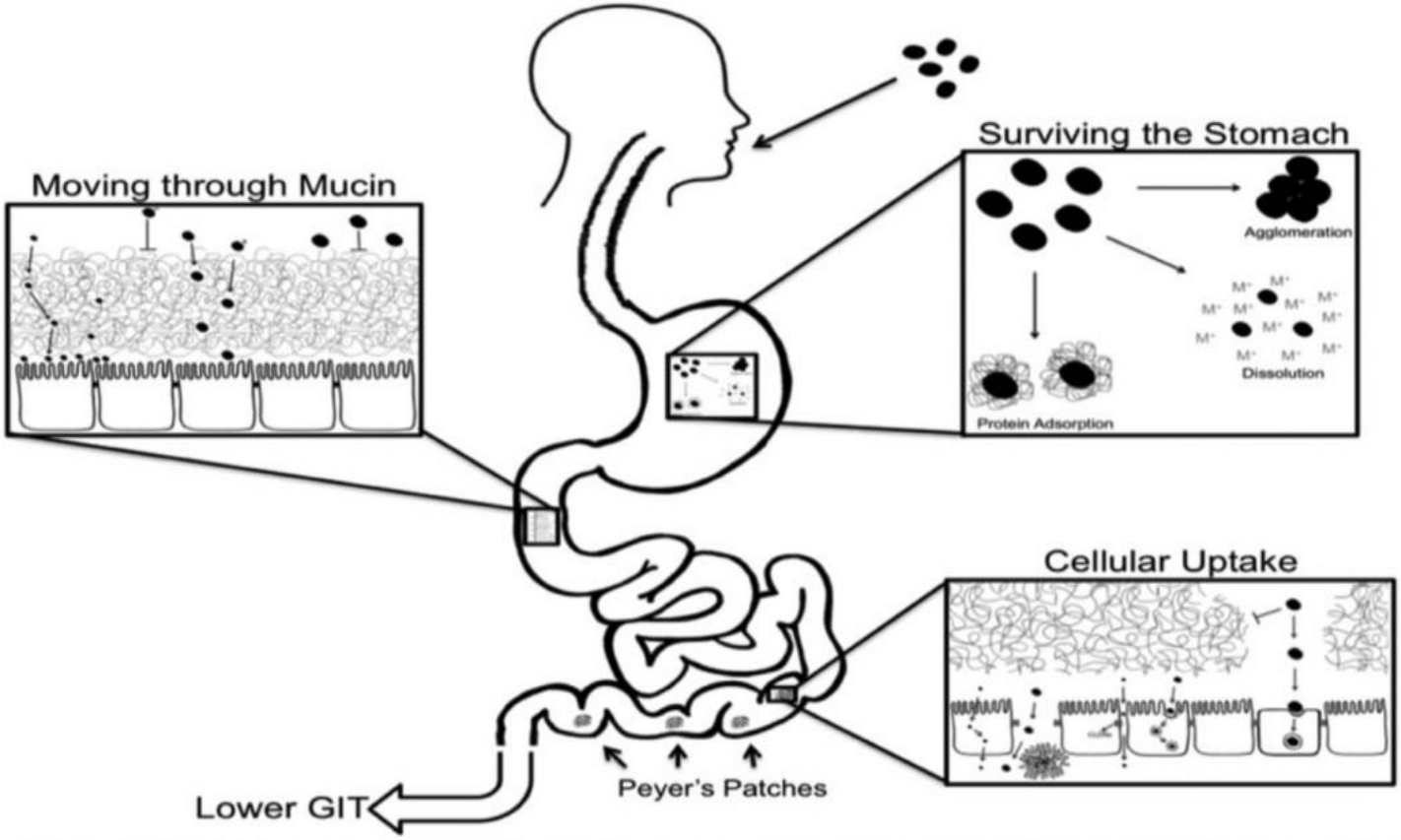


Figure 4.
Overview of barriers to gastrointestinal absorption of small particles.

D. W. Hobson et al., Int. J. Toxicol. 2016, 35, 5–16

Nanomaterials in Food

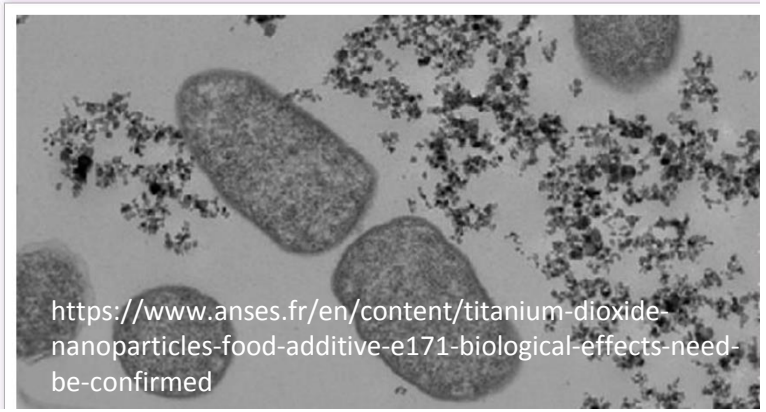
Dunkin' Donuts to remove titanium dioxide from donuts

The baked goods giant says it will remove whitening agent from its powdered donuts over fears it might contain toxic nanomaterials

▲ Dunkin' Donuts wants to remove titanium dioxide from its donuts amid health concerns. Photograph: Shawn Gay/Flickr

https://www.theguardian.com/sustainable-business/2015/mar/11/dunkin-donuts-to-remove-whitening-agent-from-donuts

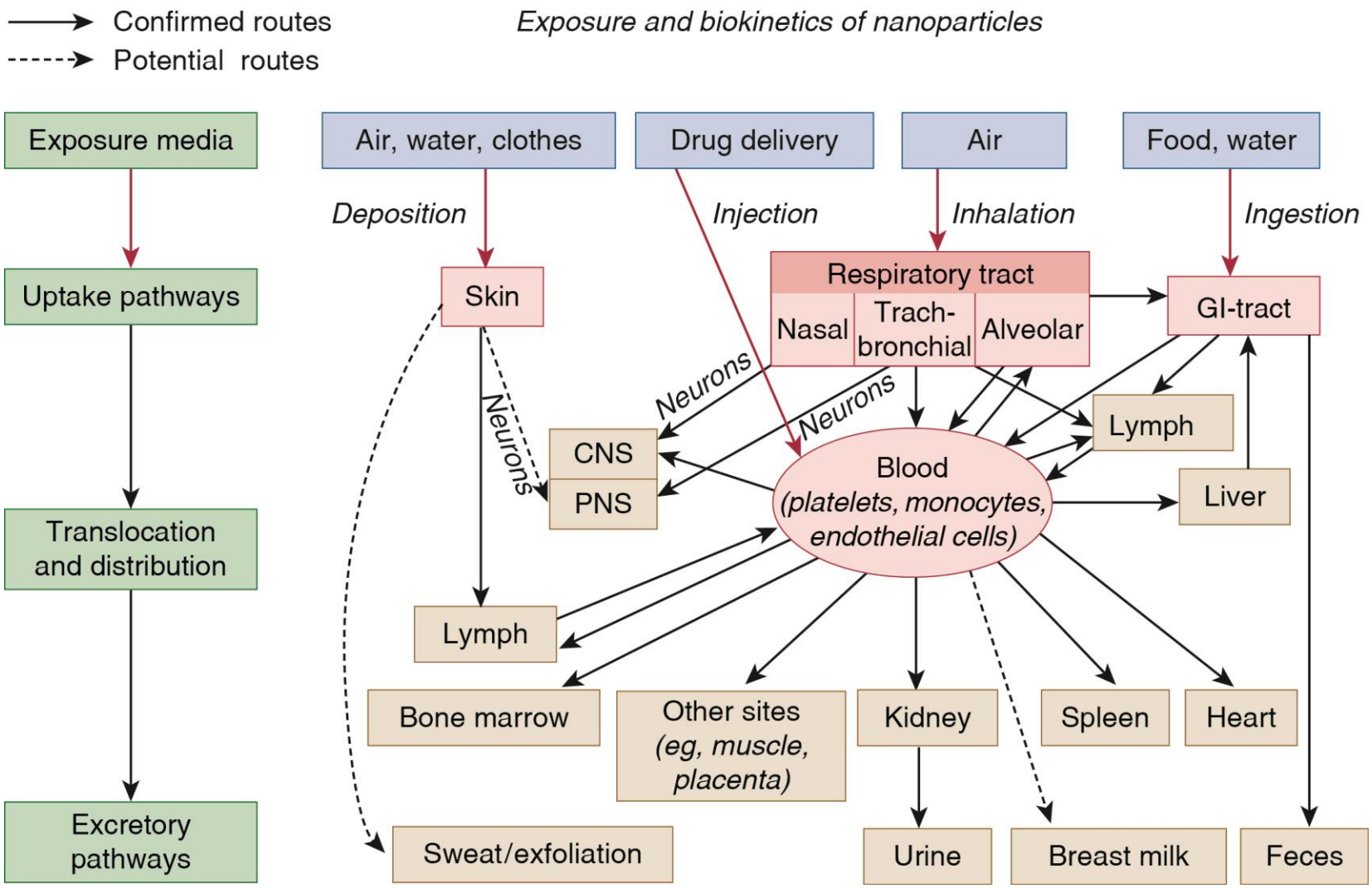
Titanium dioxide nanoparticles in food (additive E171): biological effects need to be confirmed



- Since 2007, several inorganic NPs are used in food industries and in the pharmaceutical industry as an additive: AgNPs, TiO_2NPs, ZnONPs, SiO_2NPs
- e.g. E171 (TiO_2) 1–5 µg/mg is used in sweets
- Evidences of toxicity from ingestion of NPs were found in cell culture and animal studies

V. D. Matteis et al., Toxics 2017, 5, 29

Exposure and biokinetics of nanoparticles

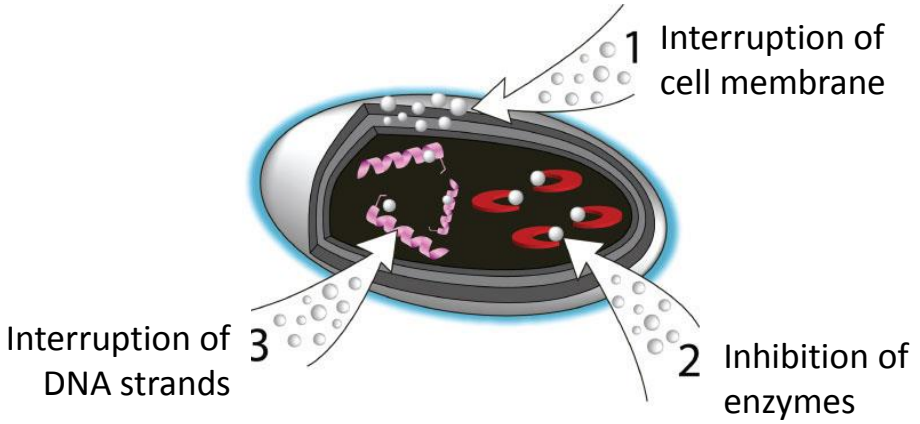


Casarett & Doull's Toxicology, 8th Ed.

Case-studies: Biological Effects of ENMs

Silver nanomaterials

Silver NMs demand for new uses in textiles, plastics, toiletries and medical industries, etc.



Silver is “Potent bactericide”

1. Development of antibiotic resistant bacteria?
2. Harmful to beneficial bacteria which form symbiotic relationship to plants, animals and humans → Disrupt ecosystem function?
3. Long term adverse effects to human?

- WHO considered silver to be a **toxic substance**
- US EPA declared silver to be **pesticide** in 1954

How might the people be exposed to silver?

Most people are exposed daily to very low level of silver mainly in food and drinking water, and less in air.

- Less than 0.001 ppb in **air**
- 0.2-2.0 ppb in **surface waters**
- 0.20-0.30 ppm in **soils**

A Case Report

A 56-year-old woman has had discolored skin since the age of 14

Argyria

At the age of 11 the patient was given nose drops of unknown composition for “allergies,” and three years later her skin turned gray. She was thought to have argyria, and a skin biopsy at the age of 15 confirmed the presence of silver deposition.

The facial pigmentation was diffuse until the age of 36, but it became patchy after dermabrasion. The patient has had no other related problems.

Colloidal silver products sold in the early 1900s had silver concentrations as high as 30 percent. Suspensions of silver, available now in some health food stores and pharmacies, are touted for the treatment of many disorders, including the acquired immunodeficiency syndrome, cancer, sore throats, meningitis, parasites, chronic fatigue, and acne, without substantiation.

BRUCE A. BOUTS, M.D.

New Eng J Med. May 20, 1999

Occupational Exposure Levels (OELs) for Silver

Table 1-2. Current OELs for silver in the United States and other countries

Authority	Particle Size	Form	OEL (μg/m³)	Reference
OSHA PEL*	Metal dust and soluble compounds, as Ag; total mass fraction	Soluble or insoluble	10	NIOSH [2007]
NIOSH REL	Metal dust and soluble compounds, as Ag; total mass fraction	Soluble or insoluble	10	NIOSH [2007]
ACGIH TLV	Metal dust and fume; inhalable fraction	Soluble	10	ACGIH [2001]
		Insoluble	100	
MAK†	Inhalable fraction	Silver salts (as Ag)	10	DFG [2013]
		Silver (Ag)	100	

*Current regulatory limit in the United States. †Maximum Workplace Concentration (Germany).

Reference: External Review Draft (2015) NIOSH Current Intelligence Bulletin: Health Effects of Occupational Exposure to Silver Nanomaterials

The EPA recommends that the concentration of silver in drinking water not exceed 0.10 mg/L because of the skin discoloration that may occur.

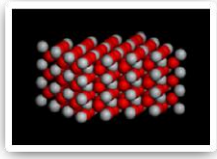
Titanate Nanomaterials

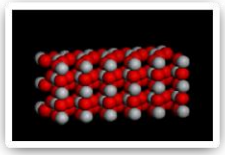
Appearance: Naturally white opaque color

Crystalline forms: Anatase, Rutile, Brookite etc.

Applications: Pigment composition in Paint, Plastics, Food additives and Health care products

TiO_2





Rutile

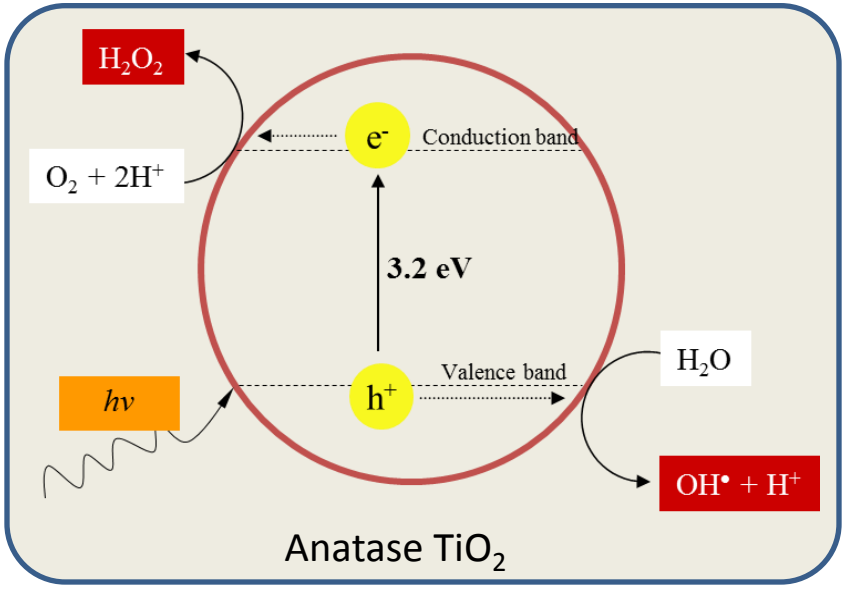
- Cosmetics
- Sunscreen products
- Food additives

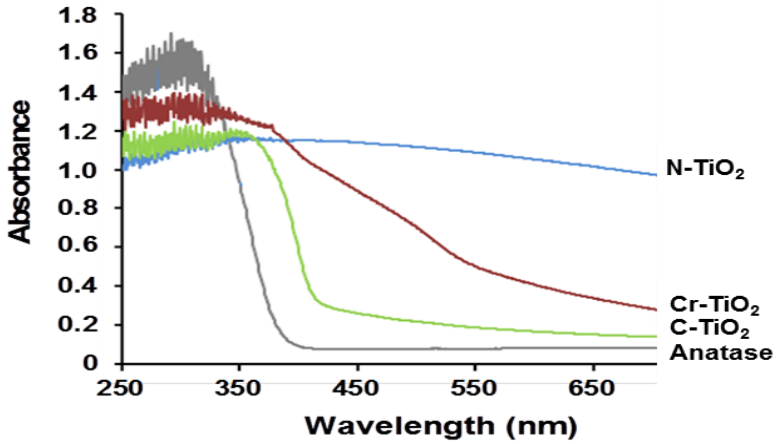
Anatase

- Photocatalytic air purification
- Self cleansing surface
- Solar energy conversion
- Self-sterilization (antimicrobial) on surface coating materials

Titanate Nanomaterials

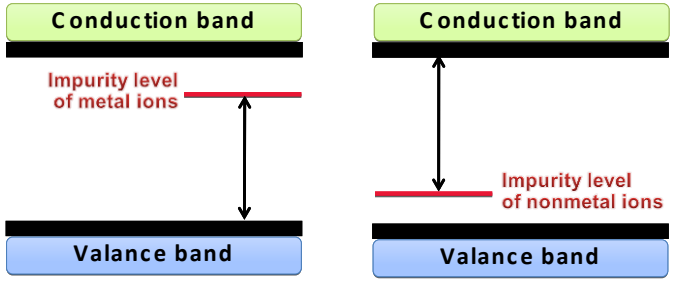
Photocatalytic activity



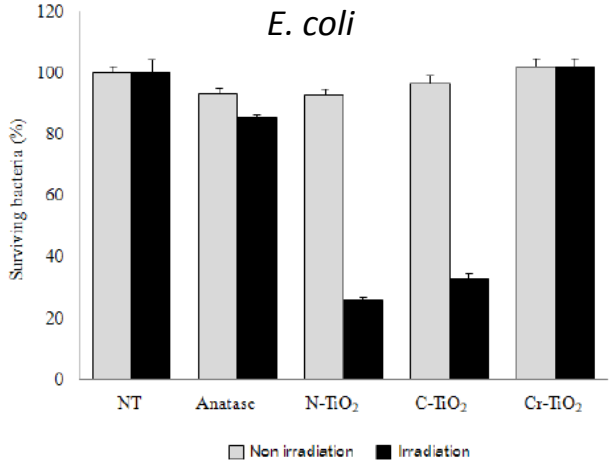


Hinthong *et al.*, Proceeding in IEEE Nano 2010

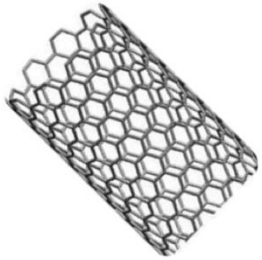
Ion-doped titanium dioxide

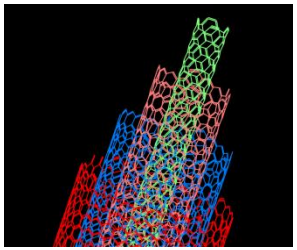


Ion-doped TiO_2	Weight fraction of phase (%)	
	Anatase	Rutile
N-TiO_2	100	-
C-TiO_2	71	29
Cr-TiO_2	20	80



Carbon Nanotubes





SWCNTs: diameter of 1-2 nm, up to 100 µm long
MWCNTs: several layer of carbon cylinders diameter of 10-30 nm

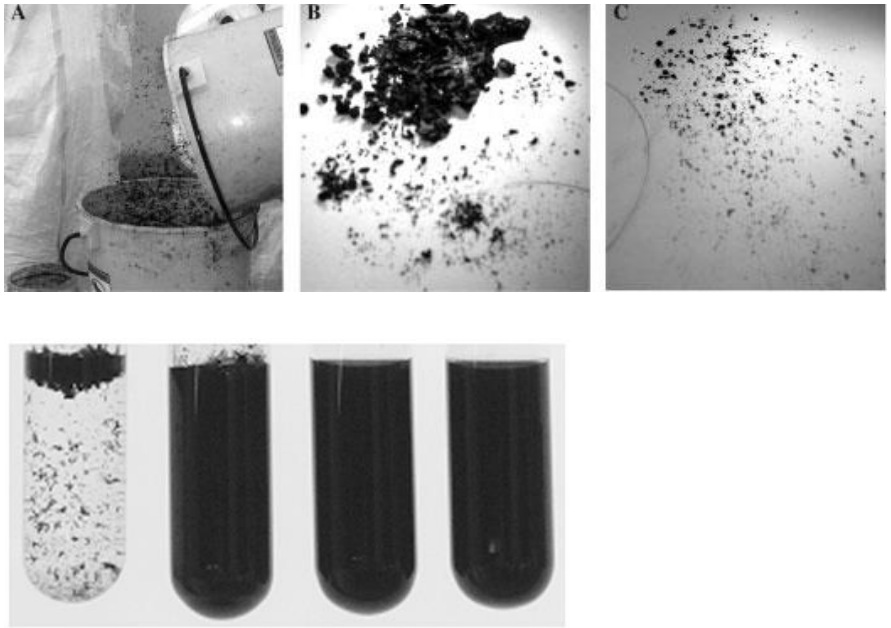
CNTs have unique properties:

- Very stable
- Tensile strength of a MWCNT was about 50 times of steel, but at a much lower weight
- Electrically insulating, semiconducting or of metallic conductance, depending on the way they are manufactured.

Applications:

- Additive for polymer composites
- Plastic in electronics
- Batteries
- Sport equipment
- Automotive industry
- Aerospace

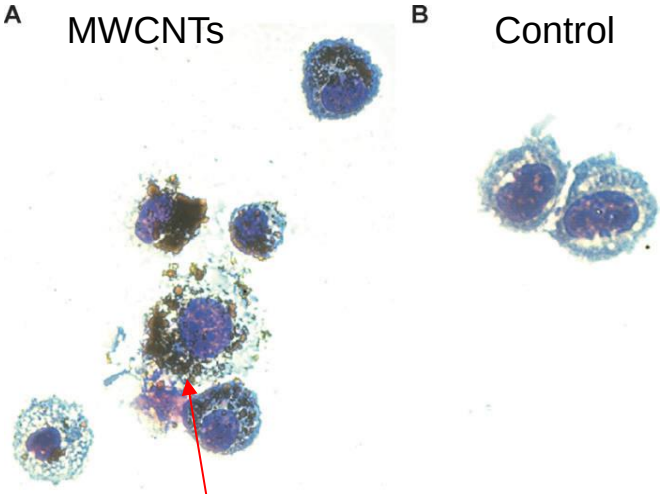
etc.



Pulmonary and Systemic Immune Response to Inhaled MWCNTs

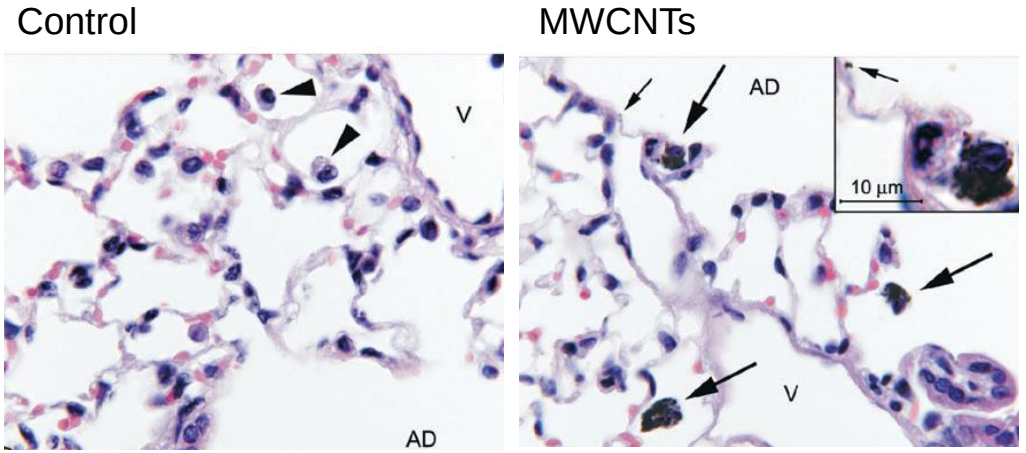
Mitchell et al. (2007), *Toxicol. Sci.* 100: 203-214.

- Male mice
- whole-body inhalation to control air, 0.3, 1, 5 mg/m^3 MWCNTs
- 7 or 14 days (6 h/day)



Representative images from BALF collected from animals exposed for 14 days to 5 mg/m^3

Many particle-laden and some enlarged macrophages



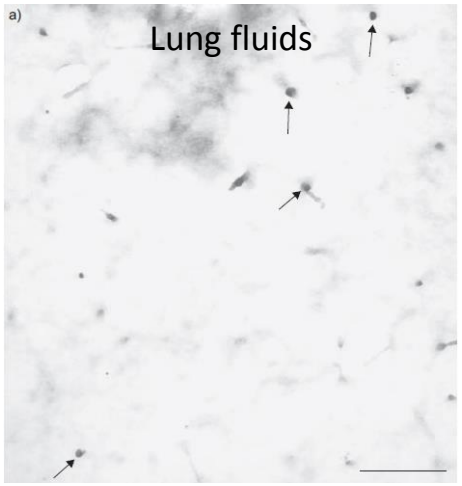
- Inhalation of MWCNTs up to 5 mg/m^3 did not cause significant lung inflammation or tissue damage
- They altered immune response functions

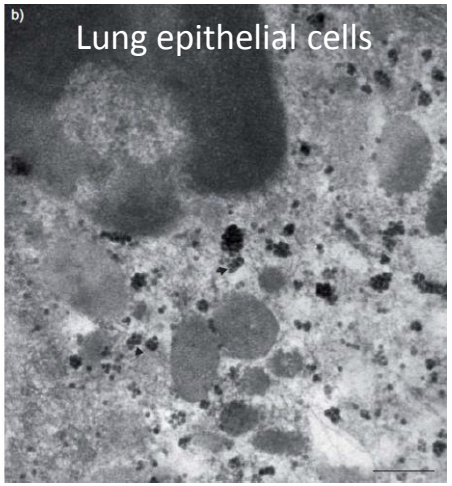
Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma

Song et al (2009), *Eur Respir J* , 34:559-567

Chinese cases

- Seven young female workers (aged 18–47 yrs.), exposed to nanoparticles for 5–13 months
- Two of them died after working for months without proper protection in a paint factory using nanoparticles,
- Their lung tissues and fluids contained nanoparticles about 30 nm in diameter
- Ball-like collections of immune cells in the lining of the lung that form when the immune system is unable to remove a foreign body.





The symptoms seen in the patients are "similar" to those seen in animals exposed to nanoparticles

Penetration and distribution of CNTs in lung: Human case

EBioMedicine
Published by THE LANCET
Volume 2, Issue 11, November 2015, Pages 1697-1704
open access

Research Article

Anthropogenic Carbon Nanotubes Found in the Airways of Parisian Children

Jelena Kolosnjaj-Tabi [a], Jocelyne Just [b], Keith B. Hartman [c], Yacine Laoudi [b], Sabah Boudjemaa [d], Damien Alloyeau [e], Henri Szwarc [a], Lon J. Wilson [c] ✉, Fathi Moussa [a, f] ✉

- Sampling from 69 asthma patients
- Particulate matter found in the alveolar macrophage cells were analyzed
- SWCNTs and MWCNTs and amorphous carbon were found, 10 - 60 nm in diameter, several hundreds nm in length
- Catalytic converters of the automobiles are manufacturing carbon nanotubes

"The concentrations of nanotubes are so low in these samples that it's hard to believe they would cause asthma, but you never know," said Rice chemist Lon Wilson, a corresponding author of the paper. "What surprised me the most was that carbon nanotubes were the major component of the carbonaceous pollution we found in the samples."

http://www.kurzweilai.net/carbon-nanotubes-found-in-cells-from-airways-of-asthmatic-children-in-paris

Published recommendations for assessment criteria

National Institute for Occupational Safety and Health (NIOSH)

Recommended exposure limits (RELs)

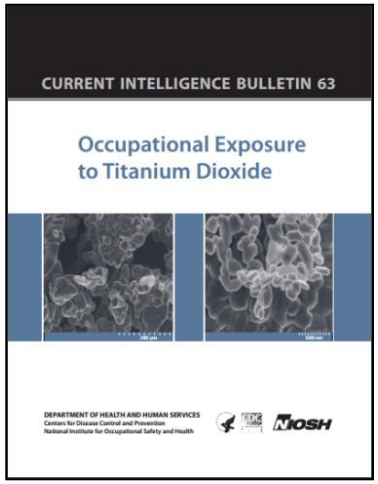
Titanium dioxide

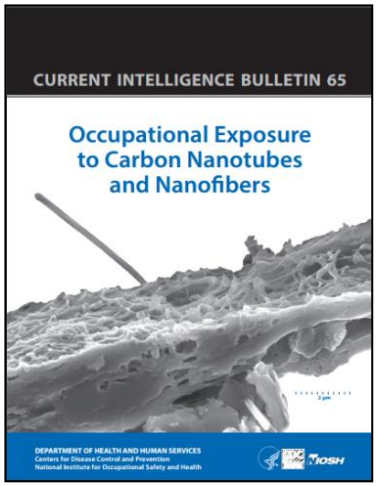
- **2.4 mg/m^3** of **fine TiO_2**
- **0.3 mg/m^3** of **ultrafine TiO_2**

  10 h/day and 40 h/week

Carbon nanotubes and –fibers

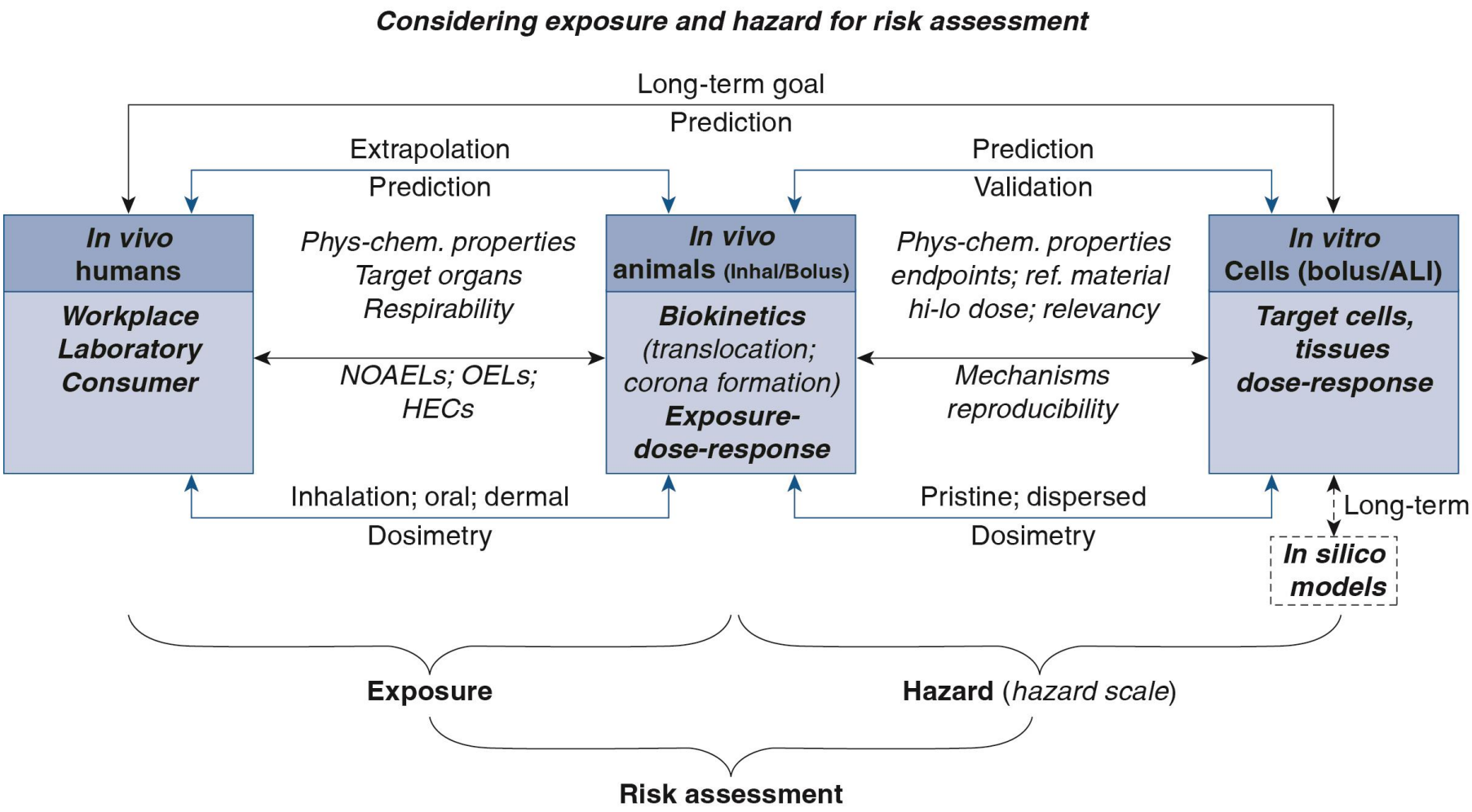
- **1 µg/m^3** of **elemental carbon as a respirable mass 8-hour TWA**





Toxicity Testing and Caveats in Nanotoxicology Assays

Concept and goals of nanomaterial toxicity testing



- Animal study can be used to predict human equivalence exposure concentration or OELs.
- *In vitro* studies are useful for uncovering underlying mechanisms of effects and toxicity ranking of nanomaterials for the purpose of hazard identification

Nanomaterial characterizations

"The first step towards nanotoxicology studies"

- To ensure that the results are reproducible
- To provide basis for understanding the properties of nanoparticles that determine their biological effects

Powder

- Purity
- Morphology
- Particle size and distribution
- Crystallinity
- Coatings
- Type of aggregation/agglomeration
- Surface properties (charge, defects, etc.)

In experimental condition

- Aggregation/ agglomeration
- Surface
- Surface coating
- Solubility
- etc.

Challenges: development of new equipment and methodology

Physico-chemical characterizations

Characterization of Nanomaterials

Analysis	Instrument
Morphology and compositions	SEM-EDX, TEM-EDX
Primary size	TEM
Hydrodynamic size, size distribution	DLS (Dynamic Light Scattering)
Surface charge	Zeta potential analyzer
Specific surface area	BET
Metal contaminants	ICP, AA

Characterization of Nanomaterials in Products → Require additional sample preparation steps such as digestion, extraction and purification etc., + advanced instruments.

Equipment for “Nano” characterization

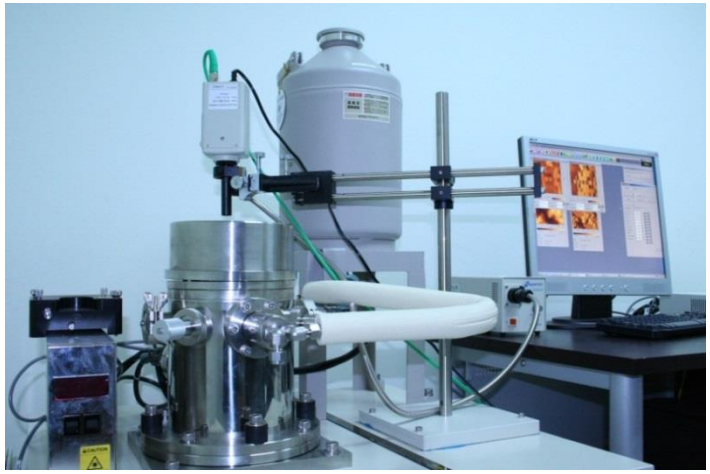
Scanning Electron Microscope (SEM)

Transmission Electron Microscope

(TEM)

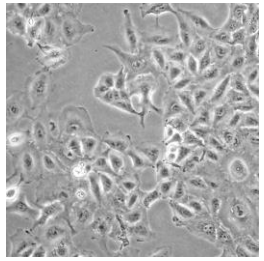
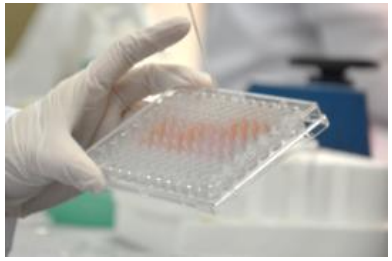
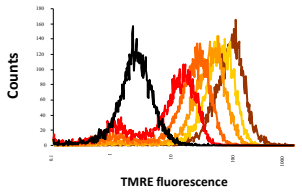
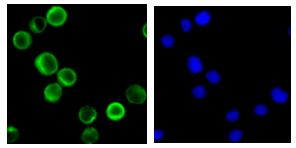
Nanosizer (DLS)

Inductive Couple Plasma Spectroscopy (ICP)



Atomic Force Microscope (AFM)

Models for toxicological studies

In vivo **Animal models**	*In vitro* **Cell-based models**
• Slow • Expensive • Labor-intensive • Ethically questionable 	• Fast • Easy • Inexpensive  
• Provide complementary data • Can be designed for exposure routes	• Exhibit abnormal behaviors • Usually aneuploid • Do not ideally represent phenotypes and mechanisms
<u>Responses:</u> • Death • Pathology (tissue, organ) • Clinical blood chemistry • Behavior	<u>Responses:</u> • Cell death • Metabolism • Gene and protein expression • Genotoxicity  

Ethical Concerns

The 3Rs Principle

Reduction

Refinement

Replacement

ข้อกำหนดการทดสอบผลิตภัณฑ์เครื่องสำอางของสหภาพยุโรป

EU Cosmetic Products Regulation (EC) No1223/2009

As of 11 July 2013

- Prohibits the testing of finished cosmetic products and cosmetic ingredients on animals (testing ban)
- Prohibits the marketing in the European Community, of finished cosmetic products and ingredients included in cosmetic products that were tested on animals (marketing ban)

Exceptions for repeated dose toxicity, reproductive toxicity, and toxicokinetics

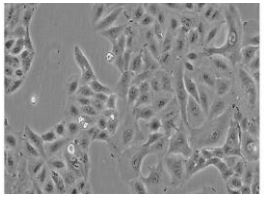
ASEAN Cosmetic Directive (บทบัญญัติเครื่องสำอางแห่งอาเซียน)

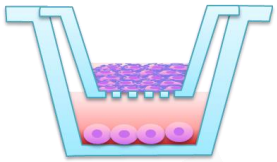
The need for alternatives to animals used in toxicity testing was recognized by **Thailand** with the ASEAN MOU signing in 2015 to adopt the directives for non-animal testing of Cosmetics. The aim of this MOU signing is to harmonize with international acceptance practices especially the EU.

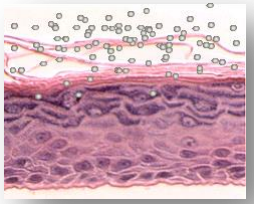
Alternative models for safety assessment

In vitro

- 2D monolayer or suspended cells
- Co-culturing of cells
- 3D tissue engineering
- Organs-on-chips

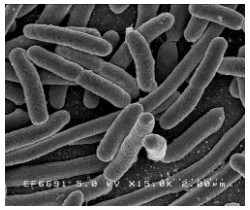


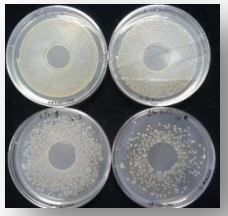




https://emulatebio.com/

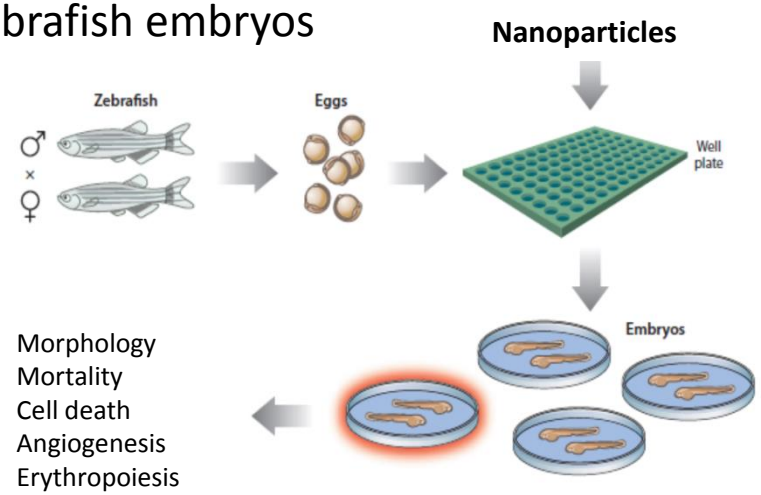
- Microorganisms





In vivo

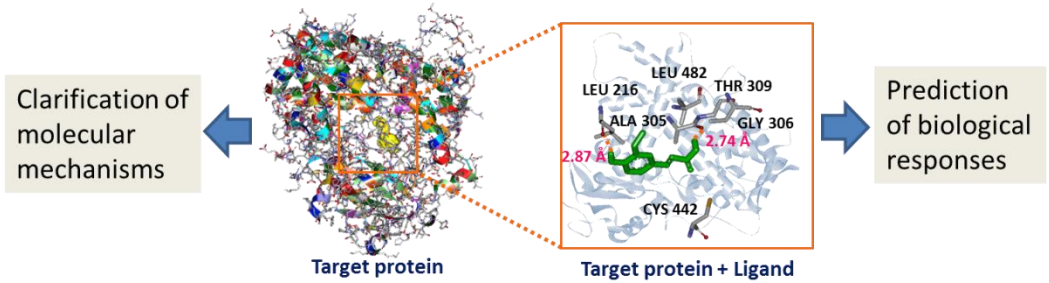
- Zebrafish embryos



Peterson, R.T. and MacRae, C.A., *Ann. Rev. of Pharm. and Tech.* 2012

In silico

- Computational simulations



Equipment for biological analysis

FACS AriaII Flow cytometry

Nanodrop

Microplate reader

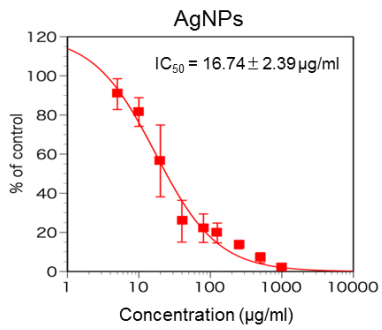
QX200™ Droplet Digital PCR

Confocal Laser Scanning Microscope (STED technology)

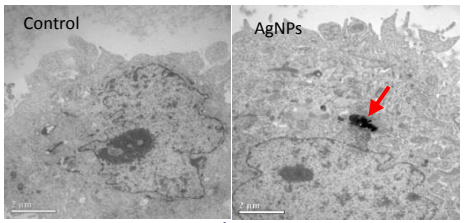
FV10i Real-time monitoring system

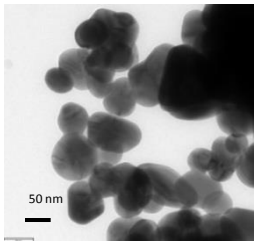
Biological Effects of Nanomaterials

Cell viability

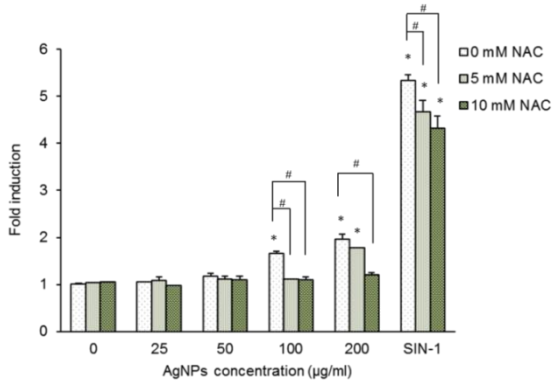


Cellular uptake

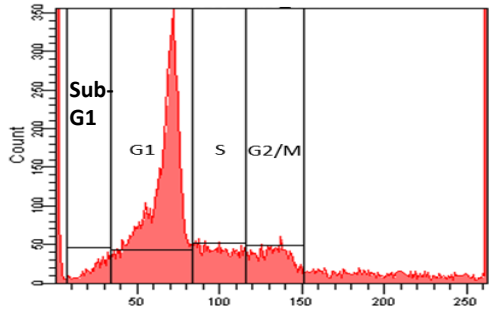




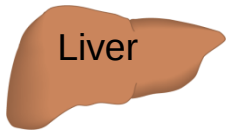
ROS generation

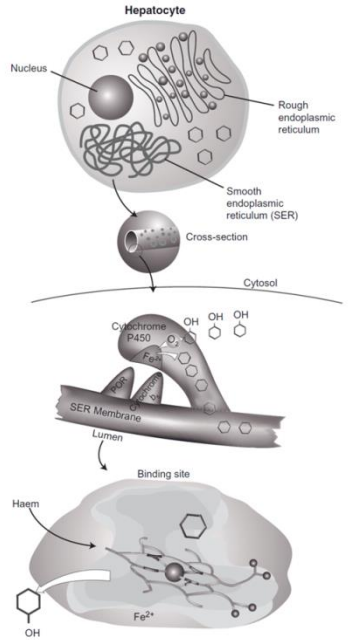


Cell cycle

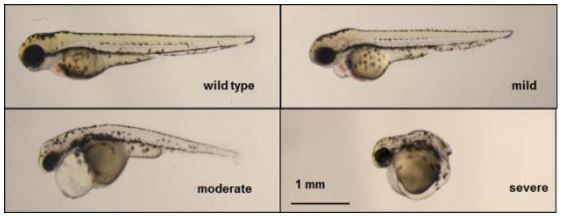


Interaction with CYP enzymes





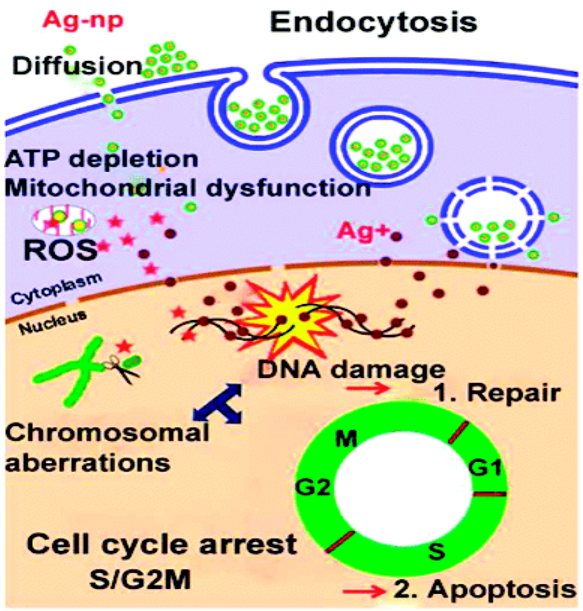
- Genotoxicity
- Expression of mRNA and proteins
- Bacterial resistance
- Embryotoxicity, teratogenicity
- etc.



In Vitro Mechanisms of Nanoparticle Toxicity

1. Damage to cell wall and plasma membrane
2. Interference with electron transport and aerobic respiration
3. Induction of oxidant stress
4. Activation of cell signaling pathways
5. Perturbed ion homeostasis
6. Release of toxic metal ions from internalized nanoparticles
7. Disruption of lysosomal membrane integrity
8. Incomplete uptake or frustrated phagocytosis
9. Interference with cytoskeletal function
10. DNA and chromosomal damage

Casarett & Doull's Toxicology, 8th Ed.

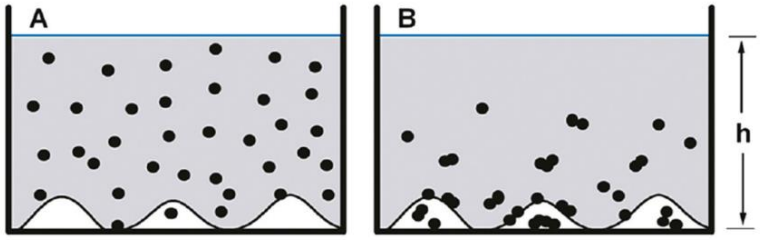


Effects of AgNPs in vitro:

- AgNPs are toxic to various cell types.
- Induction of intracellular oxidative stress. (Xia et al., 2006; Miura et al., 2009)
- Cytotoxicity of AgNPs in A549 cells was decreased by pretreatment with NAC. (Foldbjerg et al., 2010)
- AgNPs induced G2/M phase arrest in U251 and IMR-90 cells.

AshaRani *et al*. (2009) *ACS Nano*, 3:279-290.

Designed experiment for study on biological effects



The conceivable interaction of insoluble particles with submersed cells grown at the bottom of a well, filled with an appropriate medium of height h. (A) Previously employed picture, (B) more appropriate concept discussed in this study. The number of particles in (A) and (B) is the same.

Wittmaak K (2011) *ACS nano* 5:3766–3778.

Appropriate experimental design:

96- well (0.32 cm^2) → 6- well (9.5 cm^2) → Petri dish (55 cm^2)

Volume adjustment for insoluble materials

- Concentration (w/v)
- Particles per area

Caveats in nanotoxicology assays

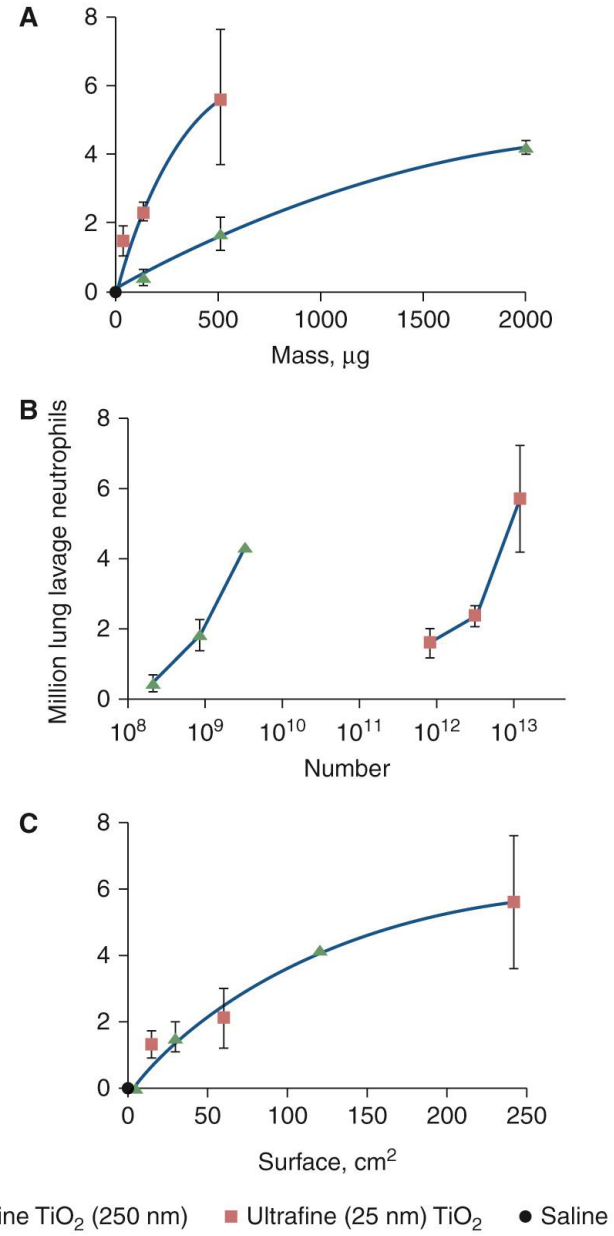
"Serious technical limitations and potentially misleading results using conventional toxicology assays"

- NPs can absorb vital dyes, cell culture micronutrients or release cytokines
- Adsorption of bacterial lipopolysaccharide or endotoxin (which can activate macrophages and dendritic cells)
- Genotoxic endpoints are sensitive to NP-induced artifacts, eg. particle agglomerates interfere with Comet assay, and cytochalasin B used in micronucleus assay interfere with NP uptake
- NP agglomerates in physiological saline may induce greater cytotoxicity than well-dispersed NPs

** In all toxicological studies, well-characterized positive and negative reference samples should be included over a range of doses and exposure times. **

Casarett & Doull's Toxicology, 8th Ed.

Dosemetrics



Pulmonary inflammatory dose-response relationships of two sizes of TiO_2 NPs induced by intratacheal instillation in rats (Oberdörster et al., 2007).

- A significant greater influx of inflammatory neutrophils into the lung was induced by 25 nm TiO_2 per **unit mass** than by 250 nm TiO_2
- There was a clear separation of the dose-response when based on **particle number** as dosemetric
- When data were expressed based on **particle surface area**, a common dose-response relationship emerged

** Identifying a biologically available surface area will be of great value for defining a proper dosemetric **

Casarett & Doull's Toxicology, 8th Ed.

Ecotoxicology of ENMs

Assessment of the environmental effects of nanomaterials

Environment	Ag [c]	ZnO [c]	TiO_2 [b]	CNTs [a]	CB [a]
Indication of hazardous effects at realistic exposure concentrations	+	+	+	--	--
Dissolution in water increases the toxic effects (++), reduces toxic effects (--)	++	++	--	--	--
Tendency for agglomeration and sedimentation (--) or no sedimentation (++)	-	-	--	--	-
Low removal rate during wastewater treatment (++), efficient removal rate during wastewater treatment (--)	-	n.a.	-	±	n.a.
Stable during waste incineration (++), burns during waste incineration (--)	+	+	++	--	--

+ : results in increased exposure or stronger effect
± : inconclusive data available
- : results in decreased exposure or lowered toxicity
n.a. : no data available

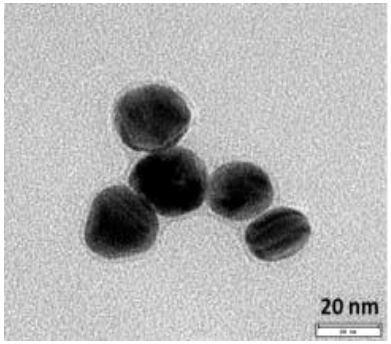
(a) Rather safe
(b) Uncertainty due to weak evidence
(c) Effects on the environment can be expected

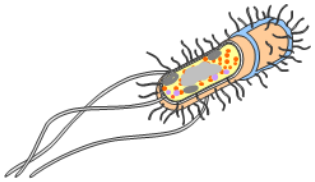
This assessment does not express the relevance of the risks of ENM versus the risks of unintentionally produced nanoscale particles from traffic.

Adapted from: Som et al., Environ Int. 2011 Aug;37(6):1131-42.

AgNPs-mediated antibiotic resistance in bacteria

Electron micrograph of AgNPs



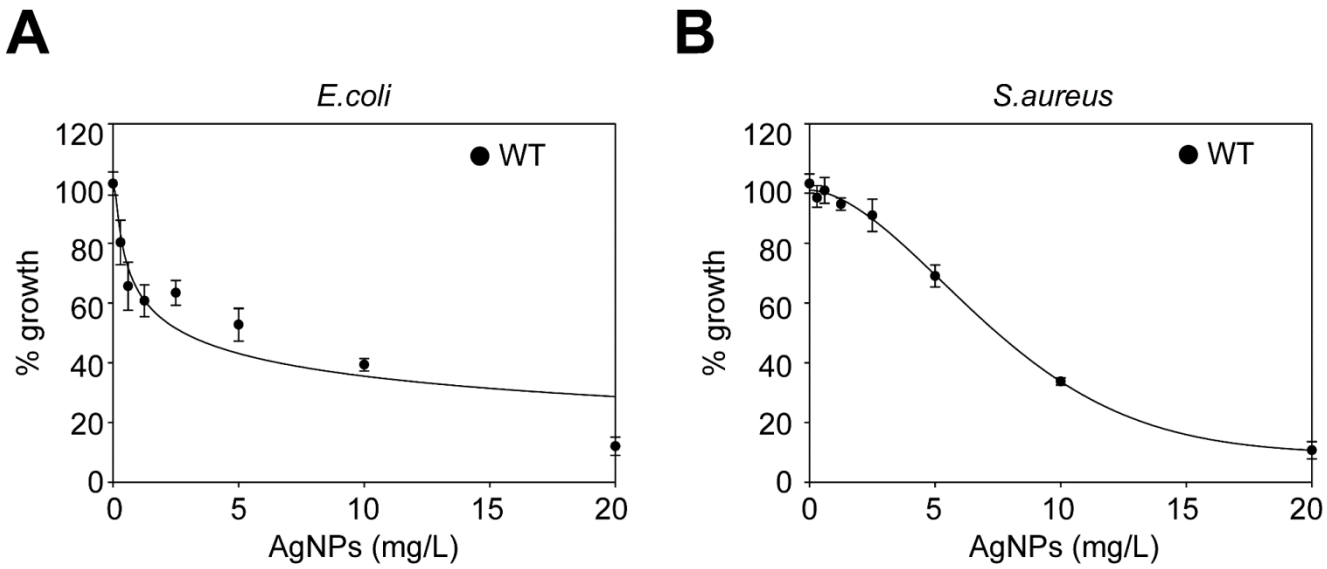


- *Escherichia coli*
- *Staphylococcus aureus*

AgNPs

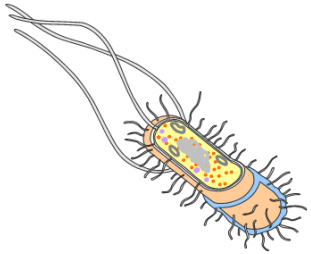
- Among highly commercialized NPs
- Global market: 1 billion USD
  (Global Market Insights: www.gminsights.com)
- Widely used as antimicrobial agents

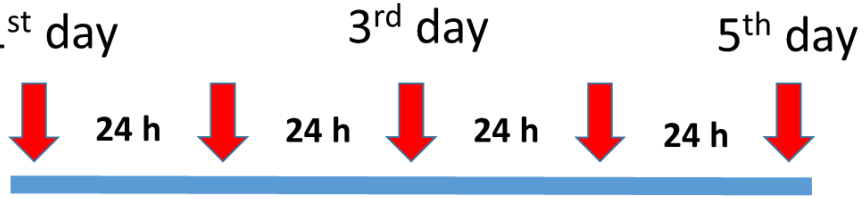
AgNPs inhibit bacterial growth in *E. coli* and *S. aureus*



Kaweeteerawat *et al.*, *J Toxicol Environ Health A.* 2017, 80:1276-1289.

Bacteria were treated with AgNPs for 5 days before selecting for surviving cells



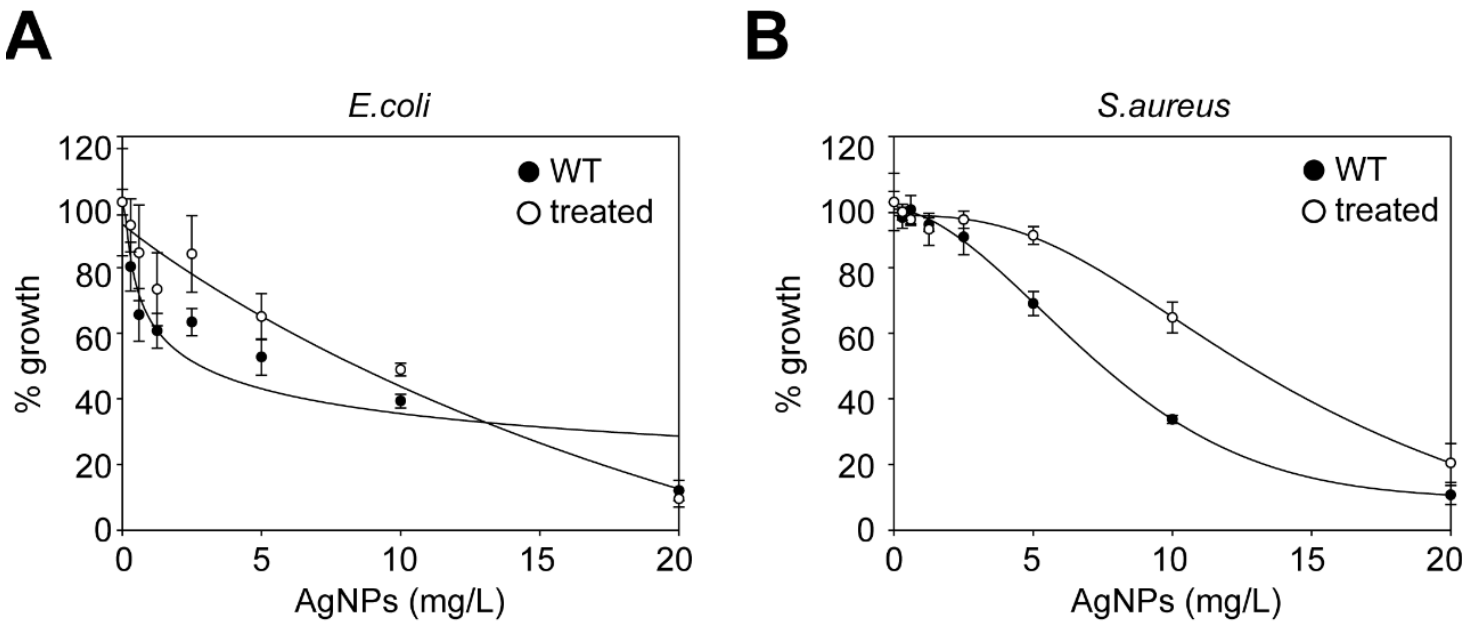


Subculture of surviving cells to a fresh AgNPs

AgNP-treated cells

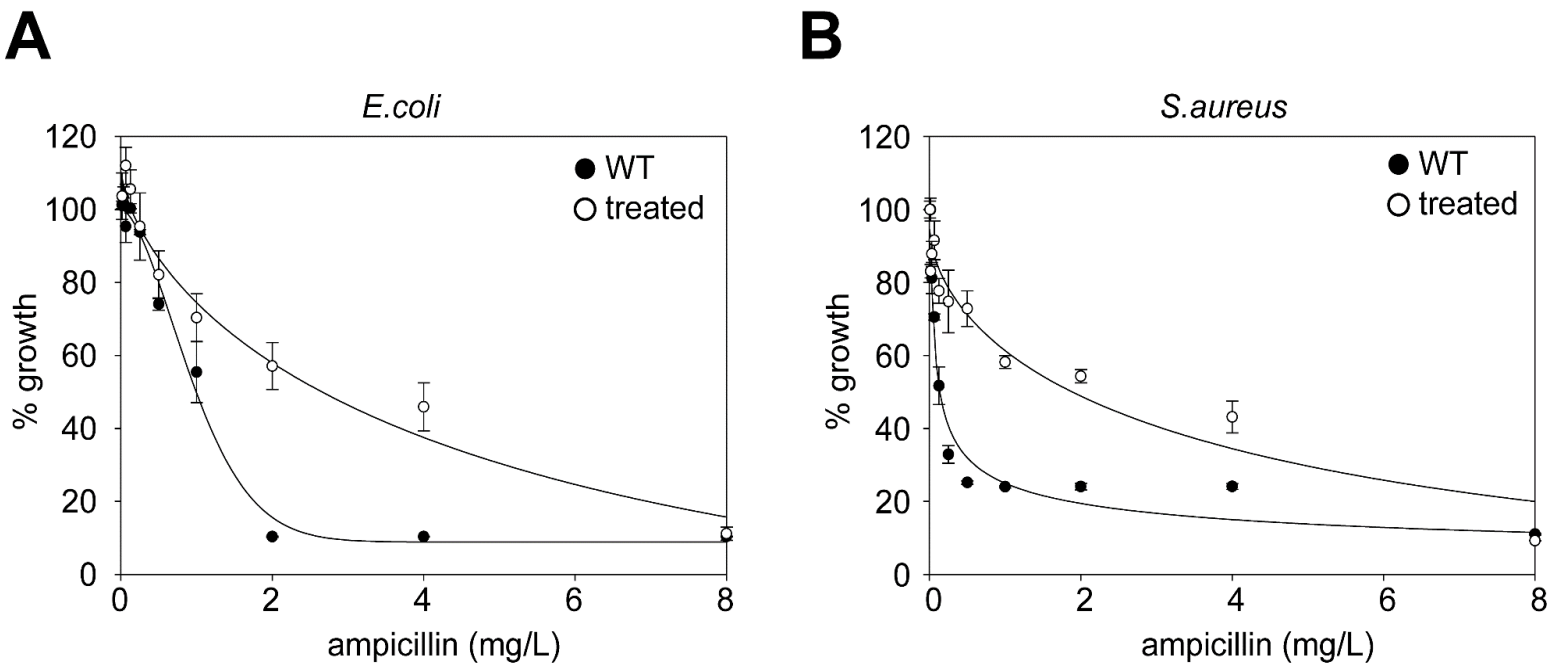
- ***Escherichia coli***
- ***Staphylococcus aureus***

AgNP pre-exposed cells exhibit resistance to AgNPs



Kaweeteerawat *et al*., *J Toxicol Environ Health A.* 2017, 80:1276-1289.

AgNP pre-exposed cells exhibit resistance to ampicillin

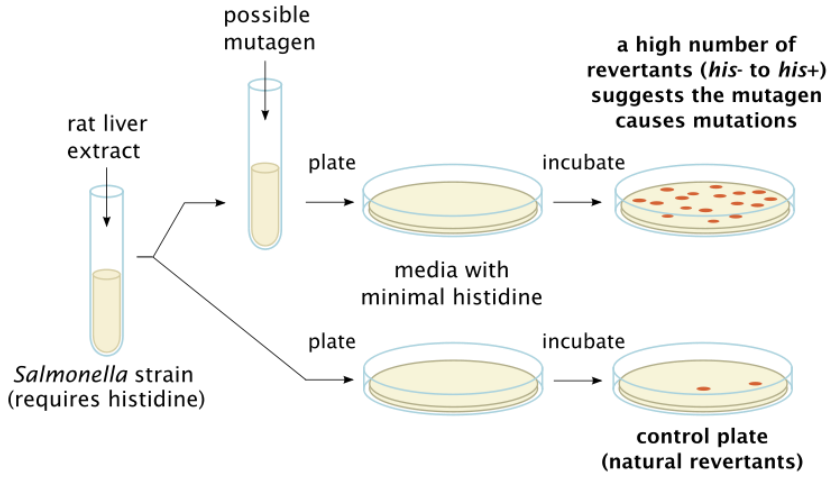


IC_{50} increase in AgNP-treated cells

IC_{50} (mg/L)	*E. coli*			*S. aureus*		
	WT	Treated	Fold increase	WT	Treated	Fold increase
Ampicillin	0.92	2.98	**3.25**	0.17	2.27	**13.25**

Kaweeteerawat *et al.*, *J Toxicol Environ Health A.* 2017, 80:1276-1289.

Bacterial reverse mutation test or Ames test (OECD 471)



https://en.wikipedia.org/wiki/Ames_test#/media/File:Ames_test.svg

Salmonella typhimurium strains	Mutational Event	Positive Control
TA1535	Base-pair substitution	Sodium azide (NaN_3)
TA1538	Frameshift	4-Nitro-o-phenylenediamine
TA98	Frameshift	
TA100	Base-pair substitution	NaN_3
TA102	All possible transitions and transversions, small deletions	Mitomycin C

AgNPs are mutagenic agents in Ames test

Number of reversed colonies	TA 1535	TA 1537	TA1538
Control	11	32	8
AgNPs 1 µg/plate	13	26	34
AgNPs 3 µg/plate	11	**485**	**627**
Positive control	309	421	430

Kaweeteerawat *et al*., *J Toxicol Environ Health A*. 2017, 80:1276-1289.

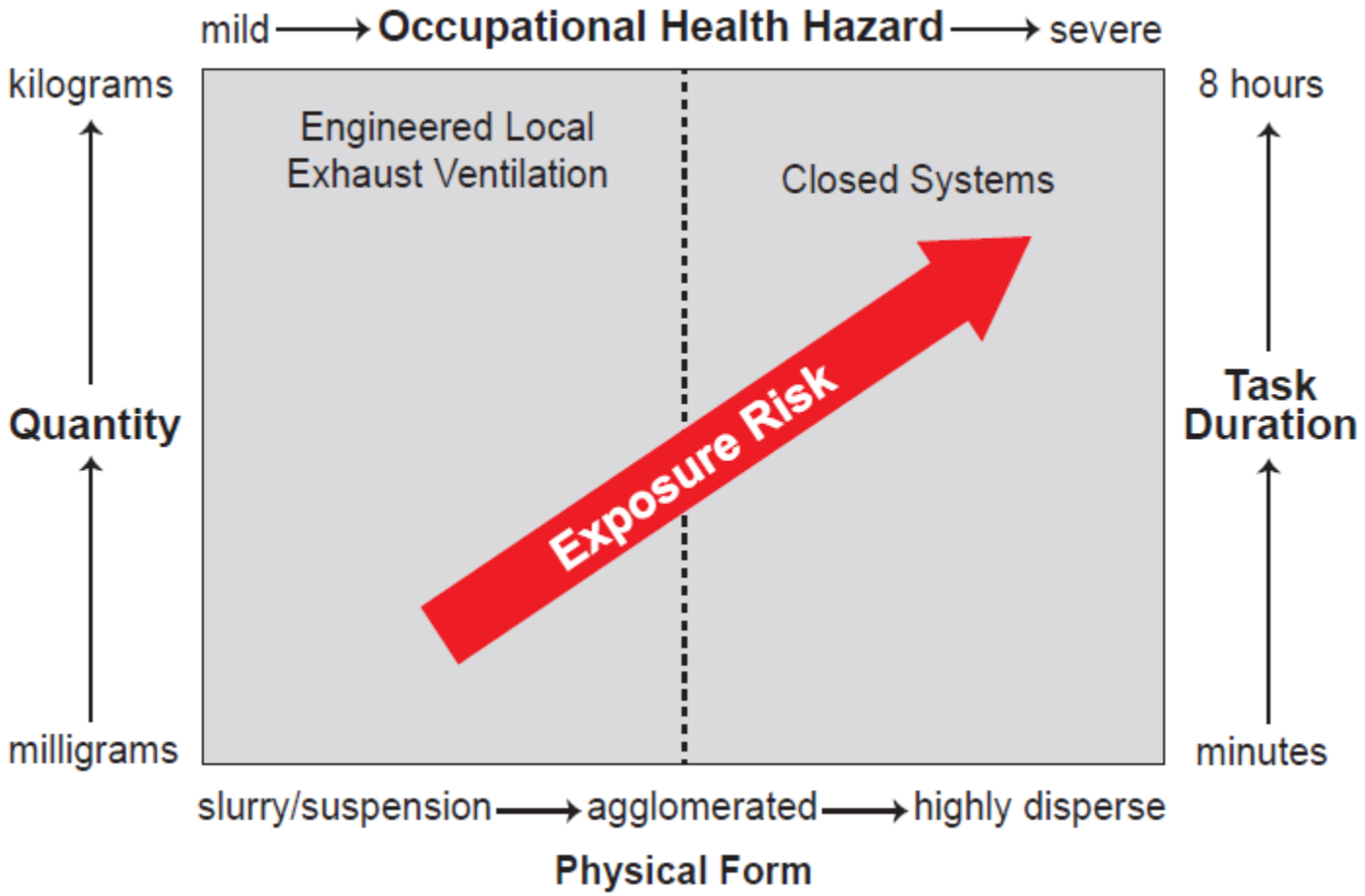
Working safely with ENMs

Hierarchy of Controls

Prevention through Design (PtD)

As companies adopt hazard control measures higher in the hierarchy, the business value is increased [AIHA 2008].

Engineering Controls

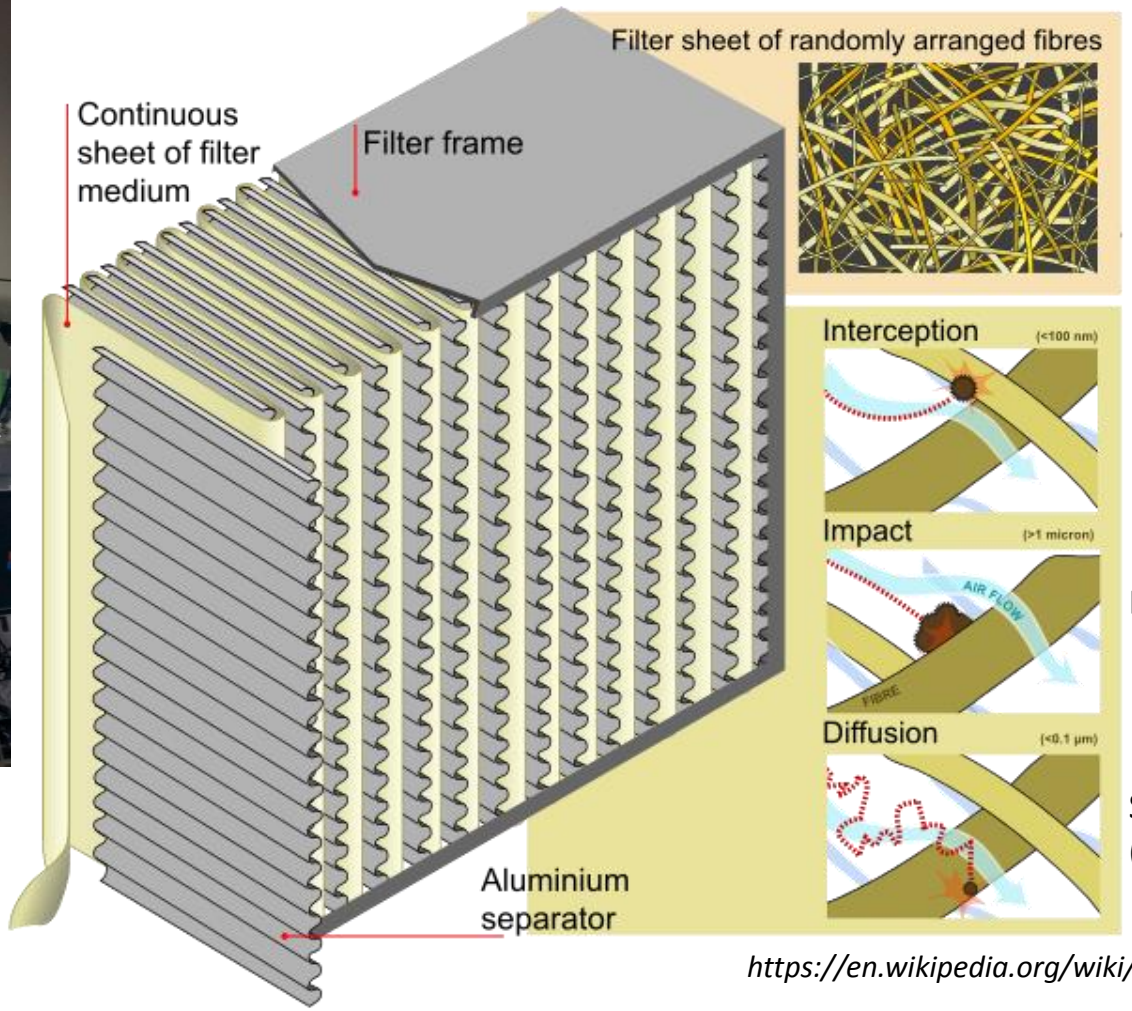


Factors influencing control selection [NIOSH 2009].

High efficiency particulate air (HEPA)

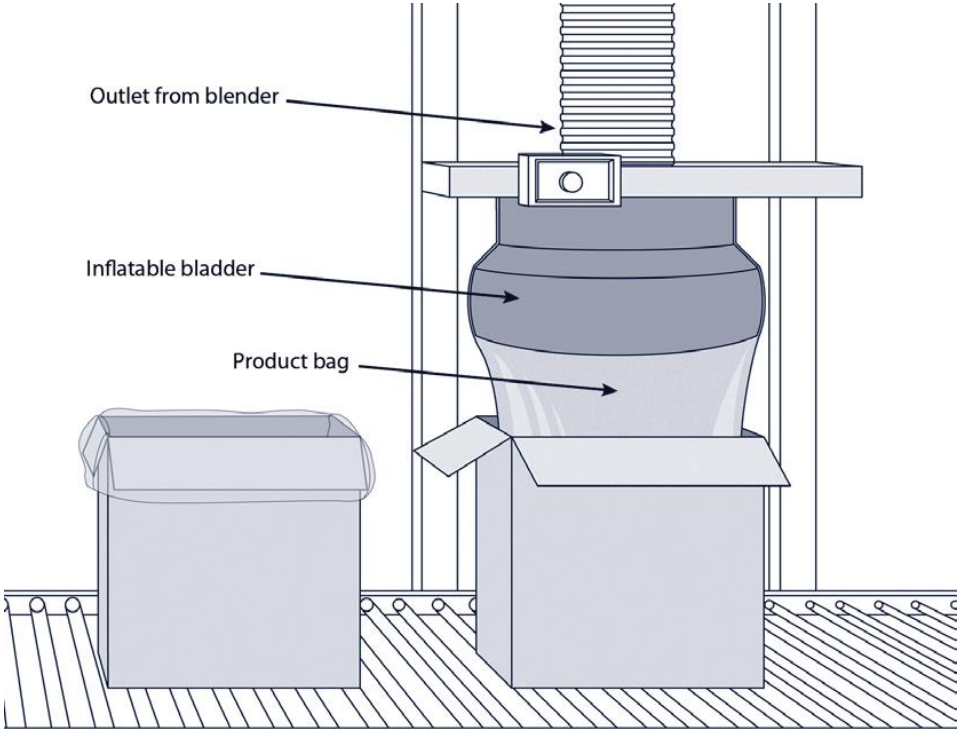
Courtesy Oak Ridge's Nanophase Materials Research facility

- Composed of a mat of randomly arranged fibers (0.5 - 2.0 µm)
- Capture 99.97% of particles ≥ 0.3 µm

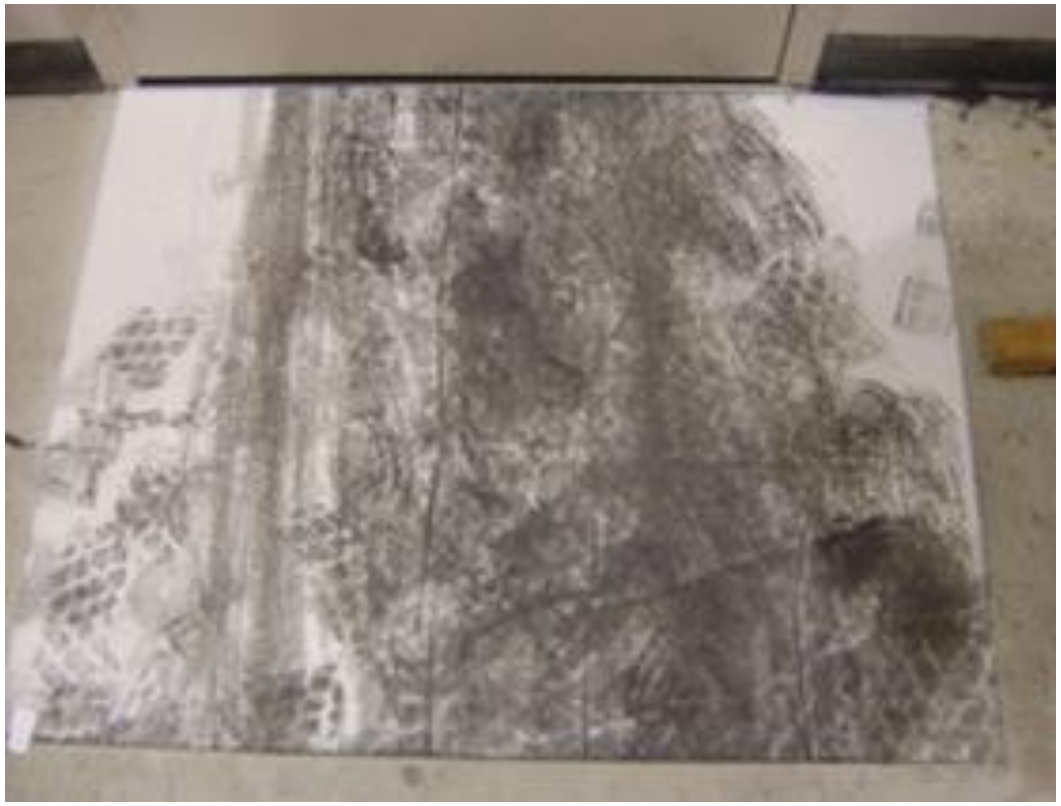


https://en.wikipedia.org/wiki/HEPA

A large-scale ventilated reactor enclosure used to contain production furnaces to mitigate particle emissions in the workplace.



An inflatable seal used to contain nanopowder/dusts as they discharge from a process such as spray drying [NIOSH 2014].



A “sticky mat,” demonstrating the collection of dirt and debris from shoes in a nanomaterial production facility.

Personal Protective Equipment (PPE)

Worker

- Avoid free air flow particles
- Maintain process containment
- Use personal protection equipment

Filtering facepiece respirators recommended for laboratory levels:
N95 and P100, FFP2 and FFP3

(NIOSH-approved) (EN certified CE-Marked)

Rengasamy et al. (2009), *Ann.Occup.Hyg.* 53: 117-128.

Masks and respiratory protection filters

***HEPA* Ventilation filters**

Different gloves

- Nitrile (most generally used)
- Neoprene
- Polyvinyl chloride (PVC)

Different gowns

Nanomaterial worker wearing personal protective equipment in a work area with local engineering controls.

Administrative controls

- Training
- Limiting access, pass keys only after completion of training
- Procedural controls
- Transport in secondary containment
- Transport in access controlled areas
- Work practices, housekeeping, labeling, storage

Source: Nano safety course, Malaysia, Nov, 2018

Cleaning and Waste Disposal

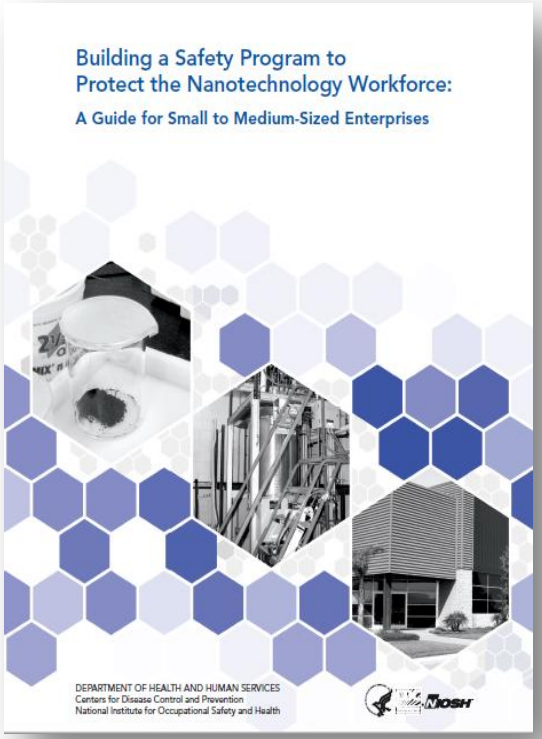
Cleaning

- Vacuum cleaning (to avoid dust explosion)
- Nanoparticles are trapped in liquid-filled drum

Specific vacuum cleaning *CEA DRT*

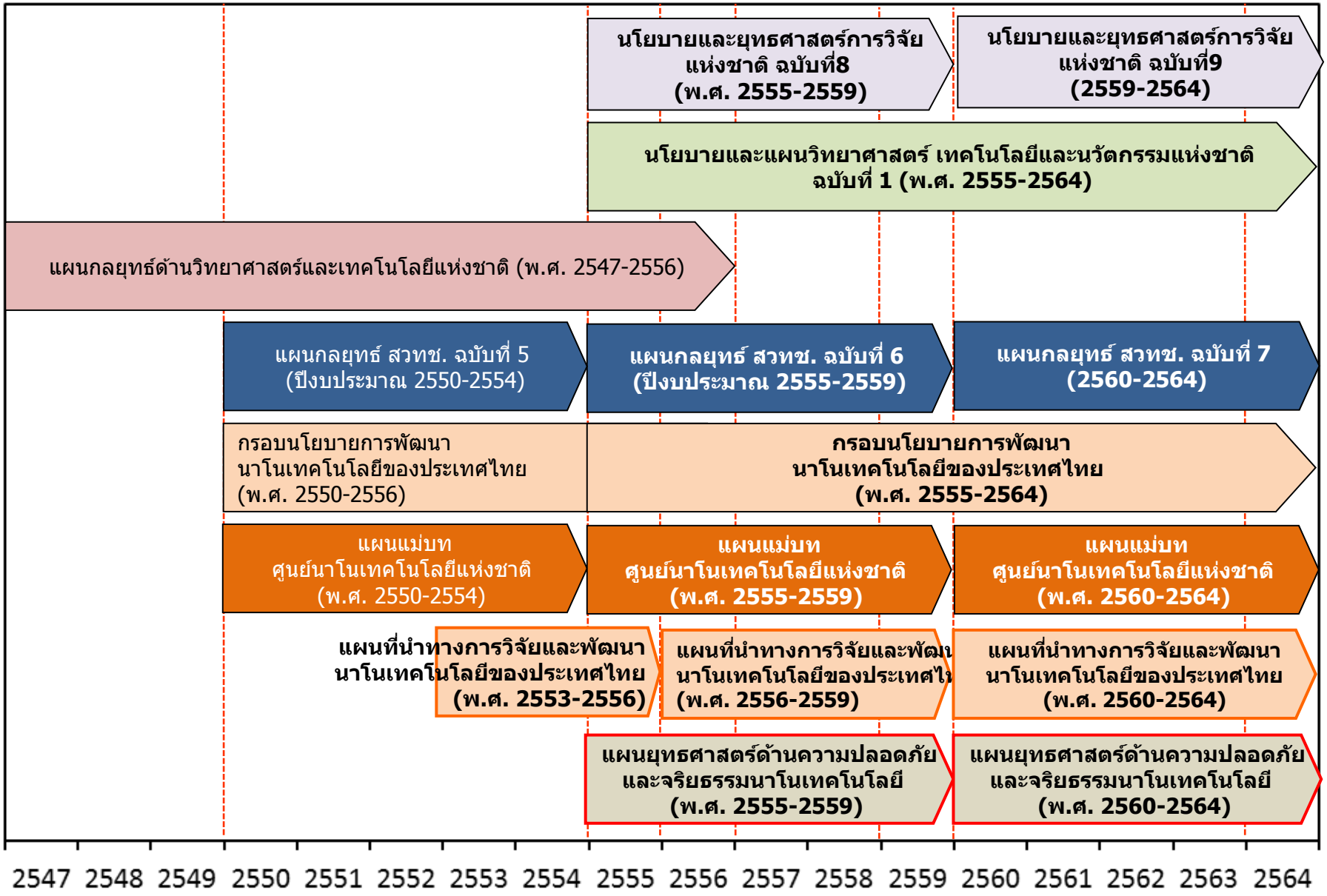
Waste Disposal

- Collect in specific drums
- Treat as hazardous waste



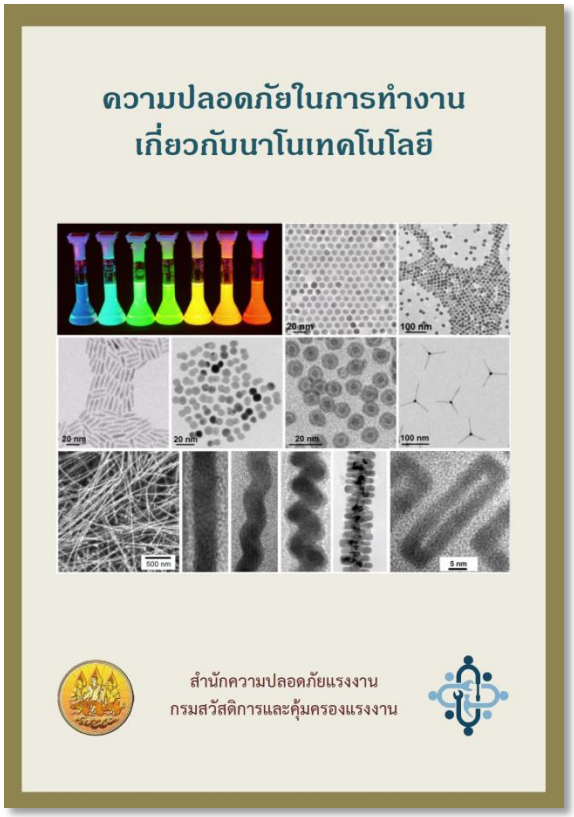
Regulatory Context

Thailand's Major S&T Plan



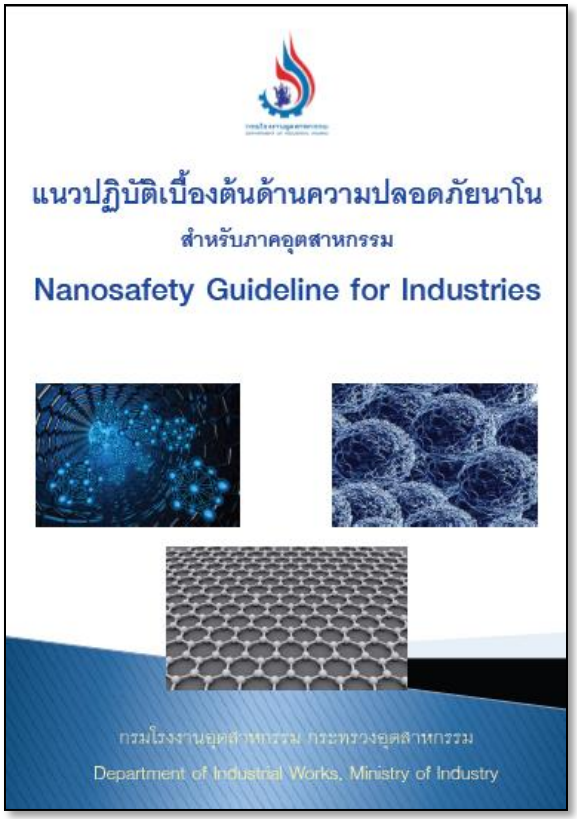
Guidance on safe handling of nanotechnology

2011



Occupational Safety and Health Bureau, Department of Labour Protection and Welfare, Thailand

2012



Department of Industrial Works, Ministry of Industry, Thailand

การพัฒนามาตรฐานผลิตภัณฑ์อุตสาหกรรม (มอก.)

มาตรฐานอุตสาหกรรมด้านนาโนเทคโนโลยี NANOTEC ร่วมกับ สมอ. ประกาศในราชกิจจานุเบกษาแล้ว 7 ฉบับ

เล่ม 1 แนวทางการระบุข้อกำหนดวัสดุนาโนจากการผลิต

เล่ม 2 แนวทางการวิเคราะห์ลักษณะเฉพาะสำหรับวัสดุนาโนจากการผลิต

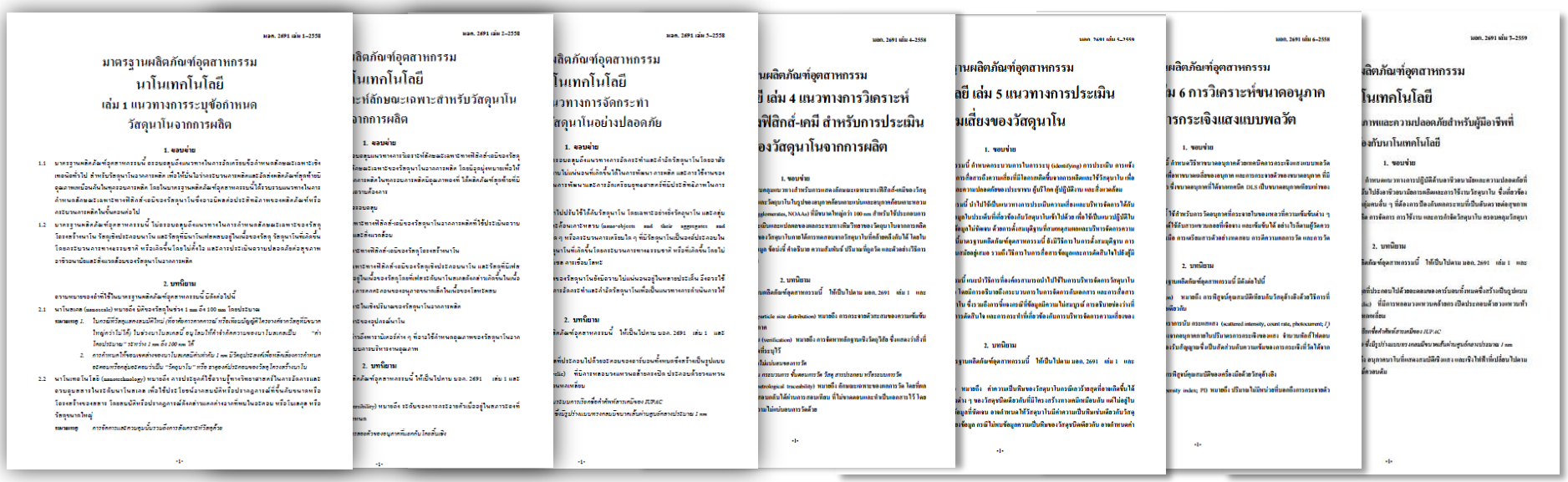
เล่ม 3 แนวทางการจัดกระทำและกำจัดวัสดุนาโนอย่างปลอดภัย

เล่ม 4 แนวทางวิเคราะห์ลักษณะเฉพาะทางฟิสิกส์ – เคมี สำหรับการประเมินพิษวิทยาของวัสดุนาโนจากการผลิต

เล่ม 5 แนวทางการประเมินความเสี่ยงของวัสดุนาโน

เล่ม 6 การวิเคราะห์ขนาดอนุภาคด้วยเทคนิคการกระเจิงแสงแบบพลวัต

เล่ม 7 วิธีปฏิบัติเกี่ยวกับสุขภาพและความปลอดภัยสำหรับผู้มีอาชีพที่เกี่ยวข้องกับนาโนเทคโนโลยี (5 สิงหาคม 2559)



แนวปฏิบัติสำหรับผู้ประกอบการผลิตภัณฑ์สุขภาพนาโน

Guidance for Industry on Nano Health Products

จัดทำโดย : คณะทำงานพัฒนาและกำหนดแนวทางการกำกับดูแลประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์สุขภาพนาโน

สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข

ประกอบด้วยสาระสำคัญ 4 บท

บทที่ 1 การนำวัสดุนาโนและนาโนเทคโนโลยีมาประยุกต์กับผลิตภัณฑ์สุขภาพ

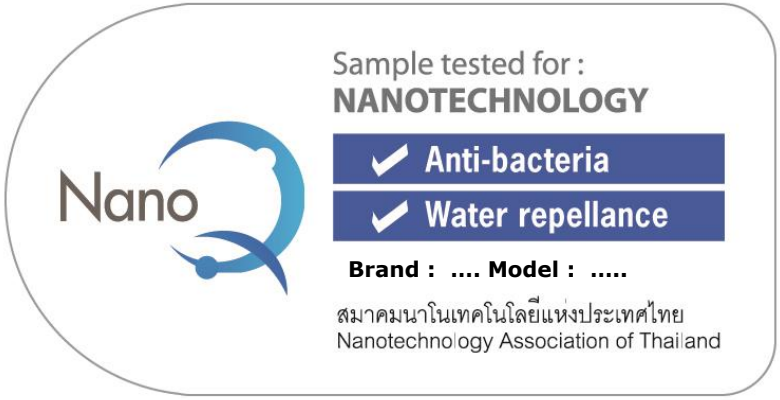
บทที่ 2 ความปลอดภัยของวัสดุนาโนและผลิตภัณฑ์สุขภาพนาโน

บทที่ 3 การกำกับดูแลผลิตภัณฑ์สุขภาพนาโนในต่างประเทศ

บทที่ 4 แนวทางการขออนุญาตผลิตภัณฑ์สุขภาพนาโนในประเทศไทย

เพื่อเป็นแนวทางสำหรับผู้ประกอบการที่เกี่ยวข้องกับผลิตภัณฑ์สุขภาพนาโนได้นำไปใช้ประโยชน์ในการศึกษาข้อมูลและจัดเตรียมเอกสารเพื่อยื่นขออนุญาตต่อสำนักงานคณะกรรมการอาหารและยาได้อย่างถูกต้อง

Nano Labeling (NanoQ)



NanoQ is a certified mark for nanoproducts (**Functional Textiles, Coating Materials, Household Products**) which are certified by Nanotechnology Association of Thailand.

Motivation to have Nano Q

- **Increase public trust** : Facilitate healthy development of nanotechnology
- **Protect consumer**: Avoid waste money
- **Protect good companies**: Eliminate unfair competitions between good and bad products
- **Facilitate trade**: Stimulate economic growth

Nano Labeling (Nano Q)

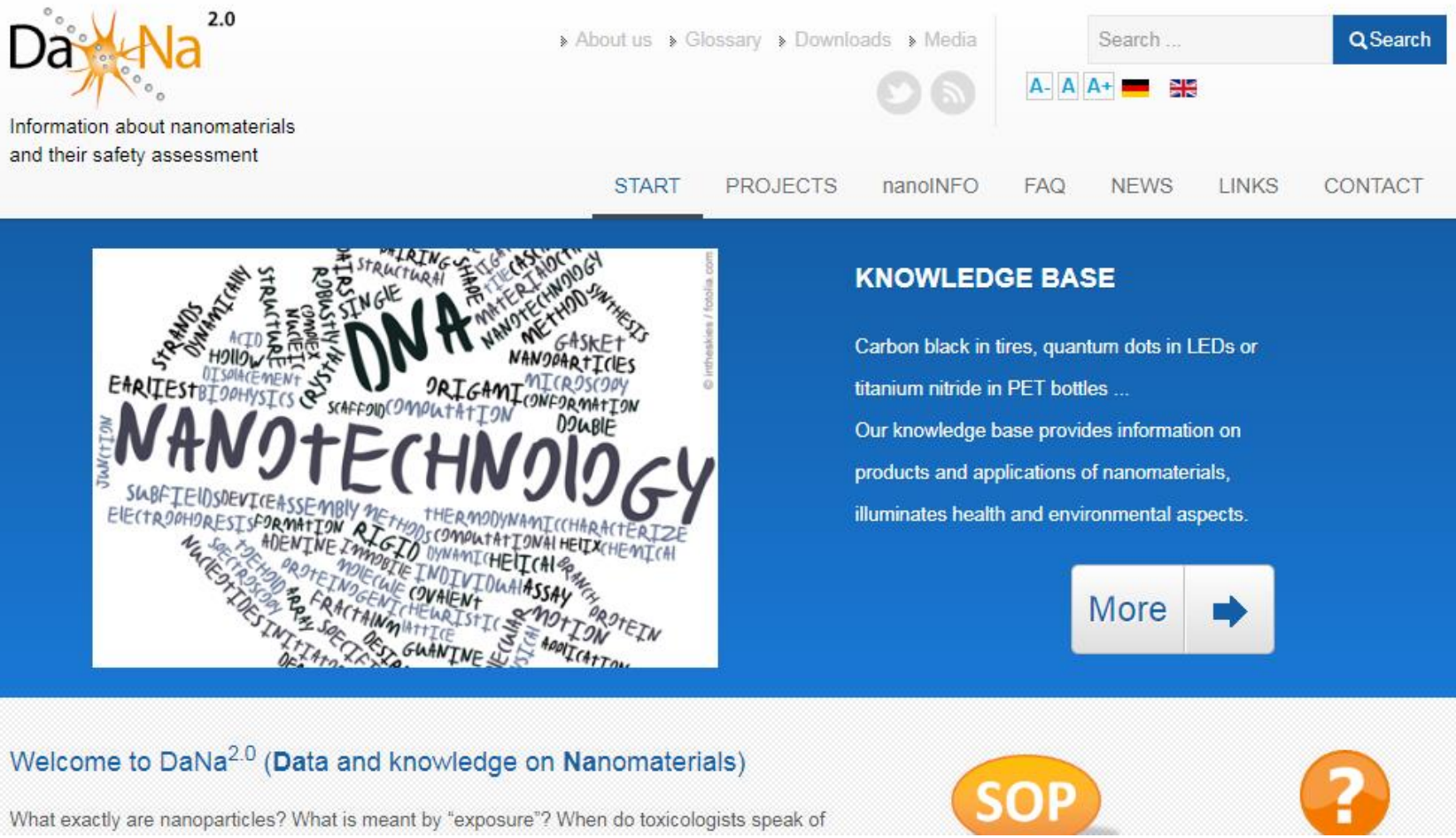
บริษัทที่ได้รับการรับรอง NanoQ

- สารเคลือบฝ้าและเพดานรถพยาบาล
- สารเคลือบผนังห้องทางการแพทย์
- ถังเก็บน้ำบนดิน
- มุ้ง
- สารเคลือบ smart coat

The aseptic ambulance of Supremeproducts Co.,Ltd has received the first NanoQ label in Thailand.

Useful Info about Nanomaterial Safety

https://www.nanopartikel.info/en/



Study services

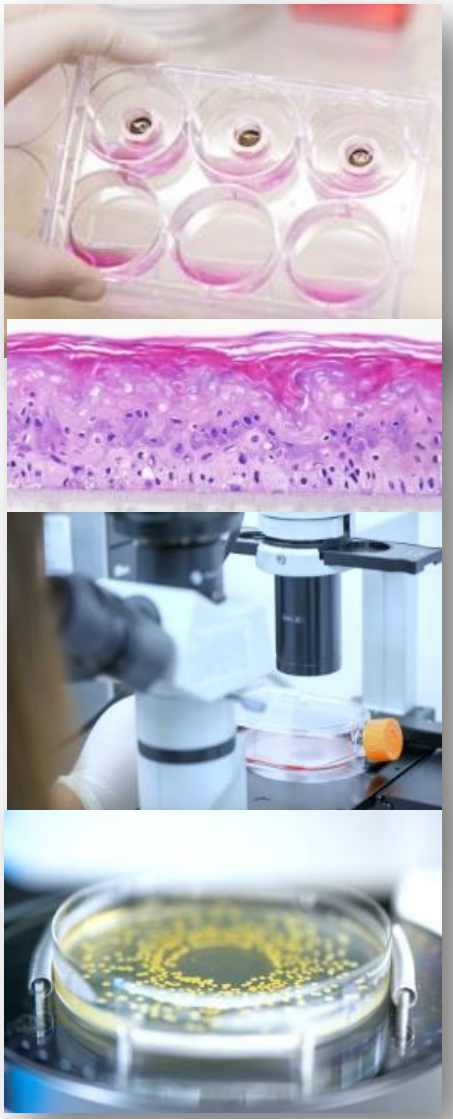
NCTC
NSTDA Characterization and Testing Service Center

สวทช NSTDA

การทดสอบด้านพิษวิทยาและฤทธิ์ทางชีวภาพ

- การระคายเคืองต่อผิวหนัง Skin irritation (OECD 439)
- การกัดกร่อนผิวหนัง Skin corrosion (OECD 431)
- ความไวต่อการกระตุ้นอาการแพ้ทางผิวหนัง Skin sensitization (OECD 442)
- ความเป็นพิษเมื่อกระตุ้นด้วยแสงยูวี Photo-induced toxicity (OECD 432)
- การระคายเคืองต่อดวงตา Eye irritation (OECD 492)
- ความเข้ากันได้ทางชีวภาพ Biocompatibility (ISO 10993)
- ความเป็นพิษต่อเซลล์ Cytotoxicity (ISO 10993-5)
- การก่อกลายพันธุ์ Mutagenicity or AMES test (OECD 471)

- ฤทธิ์ต้านอนุมูลอิสระ (Anti-oxidant activity)
- ฤทธิ์ยับยั้งการแบ่งตัวของเซลล์มะเร็ง (Anti-cancer)
- ฤทธิ์ปกป้องเซลล์จากแสงยูวี (UV protection)
- ฤทธิ์ต้านการเกิดสิว (Anti-acne)
- ฤทธิ์ชะลอวัย (Anti-aging)
- etc.

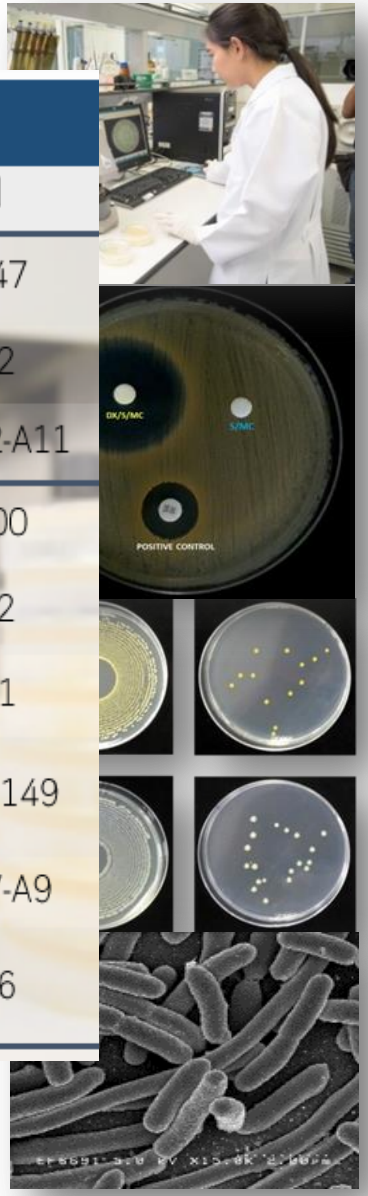


Testing services

NCTC
NSTDA Characterization and Testing Service Center

การทดสอบฤทธิ์ยับยั้งเชื้อแบคทีเรีย (Antibacterial Test)

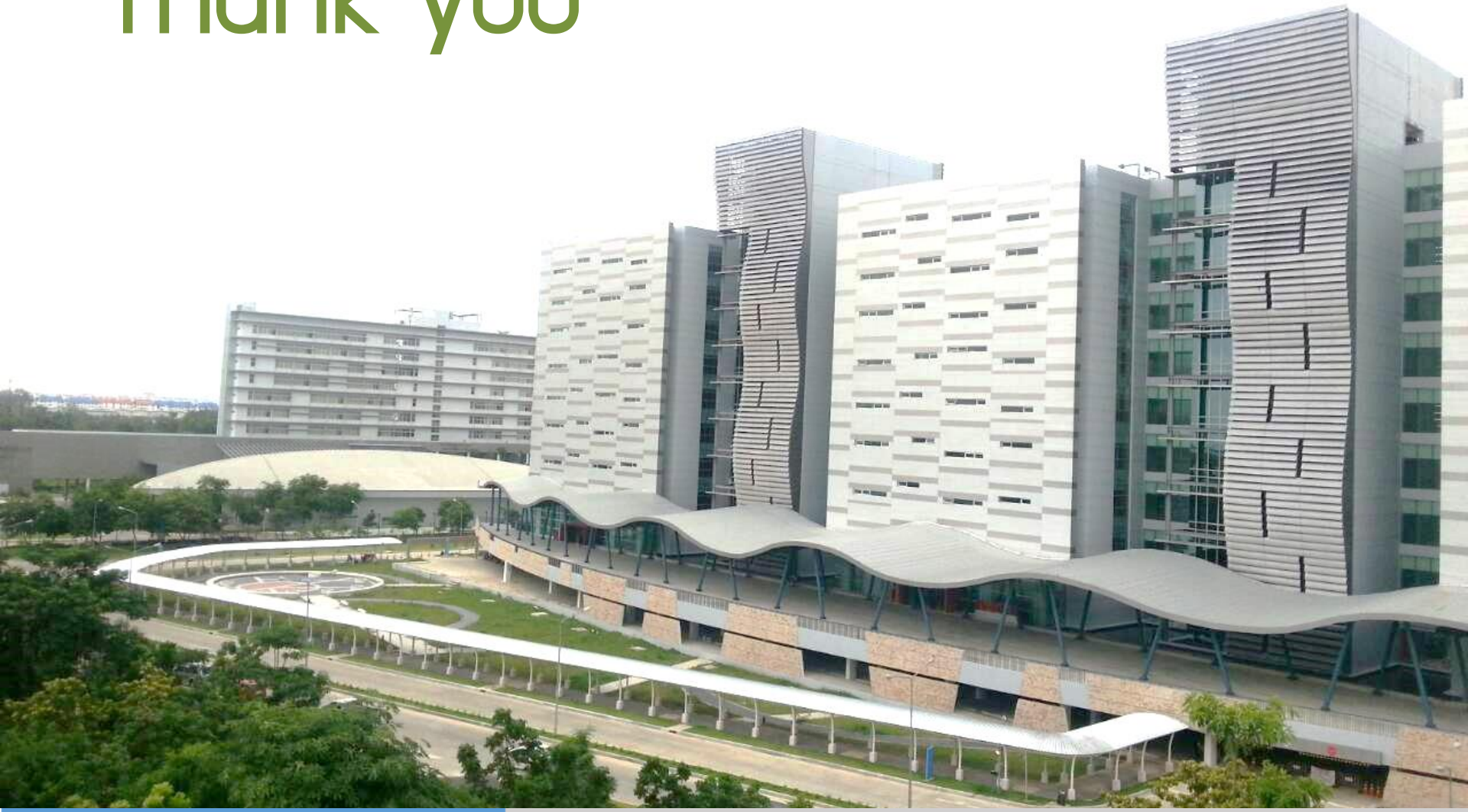
Method	Standard
• Antibacterial activity assessment of textile materials: Parallel streak method	AATCC 147
• Testing for antibacterial activity and efficacy on textile products	JIS L 1902
• Antimicrobial disk susceptibility tests	CLSI M02-A11
• Assessment of antibacterial finishes on textile materials	AATCC 100
• Testing for antibacterial activity and efficacy on textile products	JIS L 1902
• Antibacterial products -Test for antimicrobial activity and efficacy	JIS Z 2801
• Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions	ASTM E 2149
• Determination of minimal inhibitory concentrations of aerobic bacteria	CLSI M07-A9
• Measurement of antibacterial activity on plastics and other non-porous surfaces	ISO 22196



สวทช NSTDA

A Driving Force for
National Science and Technology Capability

Thank you



NCTC NSTDA Characterization and Testing Service Center

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