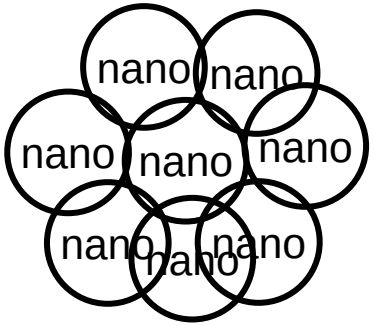
How can we evaluate health effect potency of

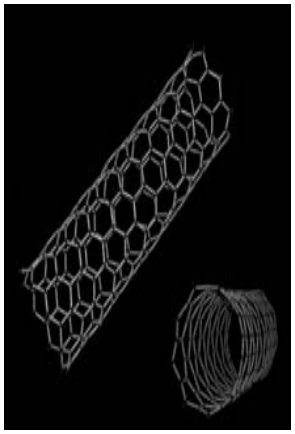


Particles?

For Session C: Human Health Effects

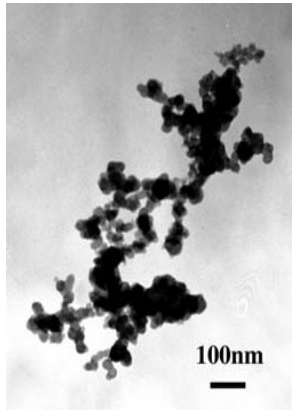
Seishiro HIRANO
Natl. Inst. Environ. Studies
JAPAN

Manufactrured and Environmental Nanoparticles



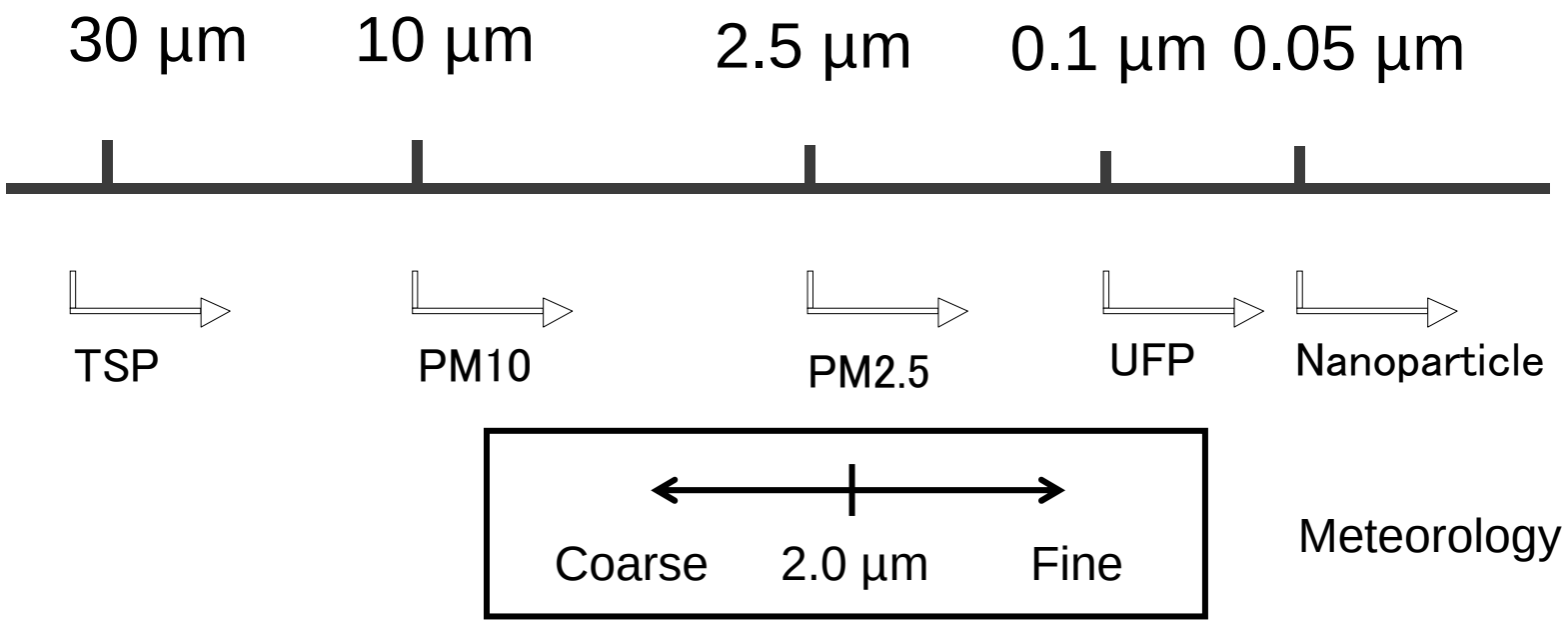
Manufactrured

may be different in nature;
and
we need to define those particles in a separate way;
but
How are they different?



Environment

Size and definition of atmospheric particles



TSP: Total Suspended Particles

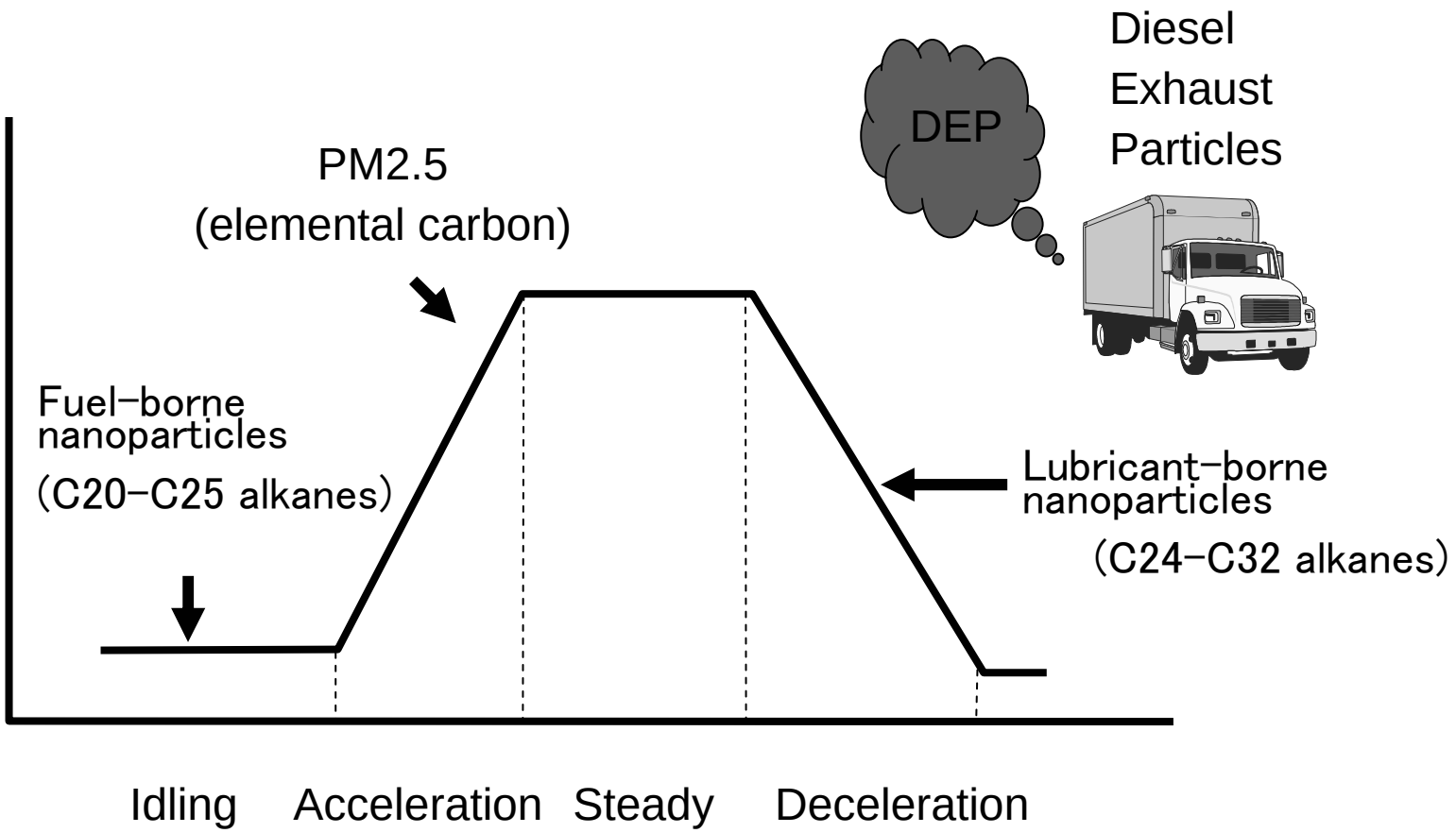
PM10, 2.5: Particulate Materials less than 10 , 2.5 µm

UFP: Ultrafine particle Nano: Particles less than 50 nm

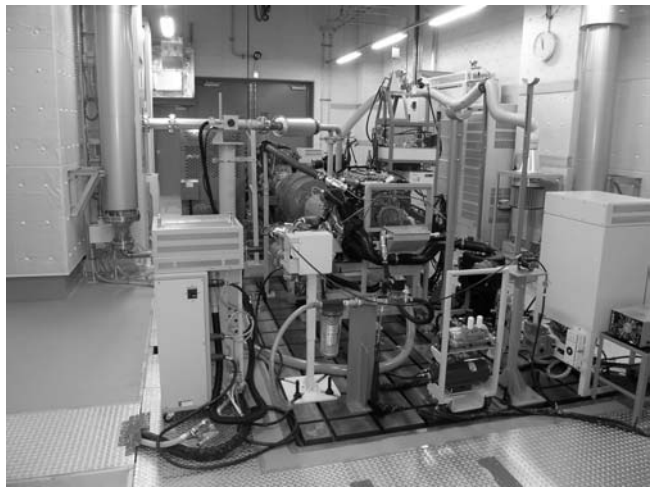
Environmental Nonoparticles

are

generated from idling and decelerating automobiles

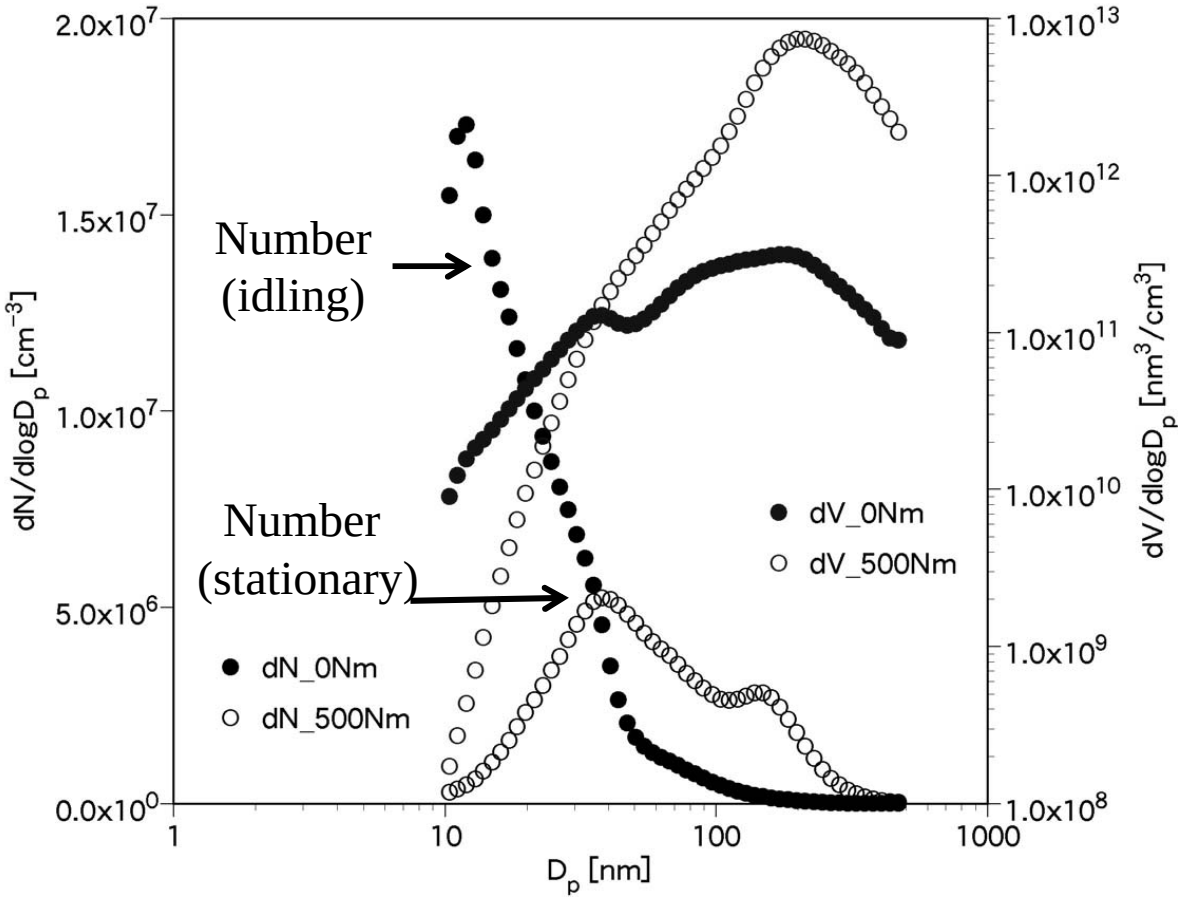


Nanoparticle Health Effect Laboratory at NIES (Tsukuba, Ibaraki)



Size distribution of DEP at stationary driving (open circle) and idling modes (closed circle)

Black circle, Number distribution
Blue circle, Volume distribution



Results with SMPS(DMA) by Y. Fujitani

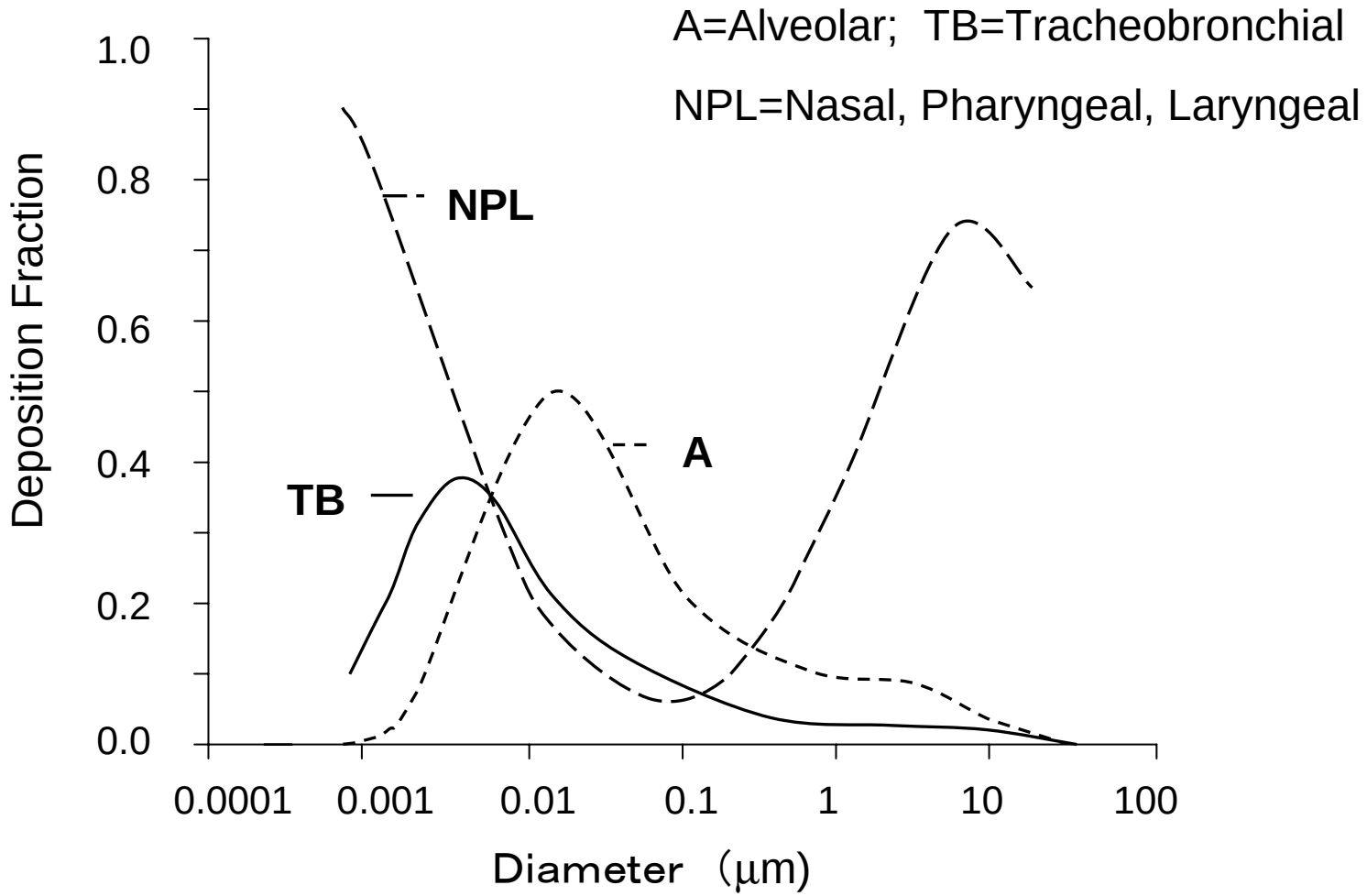
Conceptual Difference in Nanoparticles between Environmental & Manufactured

	Environmental	Manufactured
■ Size	■ ≦50nm (≦100nm: Ultrafine)	■ 1～100nm
■Dimension	■ 2 of the 3 dimensions (?)	■ 1 of the 3 dimensions
■ Name	■ Nanoparticle ■ Fibrous respirable particle	■ Nanoparticle, Nanoegg ■ Nanotube, -rod, -fiber, -wire, -rope, … ■ Nanosheet
■Component	■ Carbon soot ■ Hydrocarbons (Alkanes) ■ Heavy metals, sulfur	■ Carbon (fullerene, nanotubes) ■ TiO_2, (ZnO ?) ■ CdSe, Metalloids, Transition metals
■ Source	■ Automobile exhaust ■ Friction ■ Catalyst	■ Manufacture of nanomaterials ■ Friction

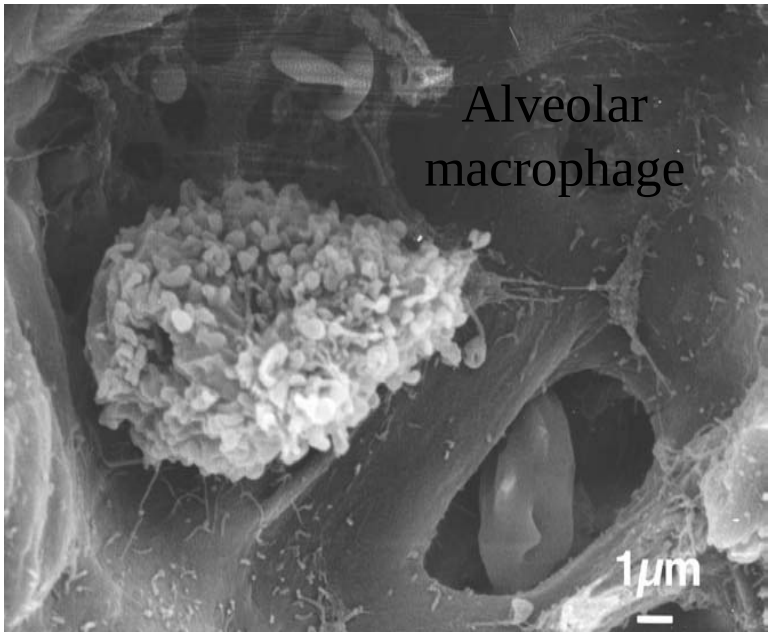
Nanoparticles and >100 nm particles

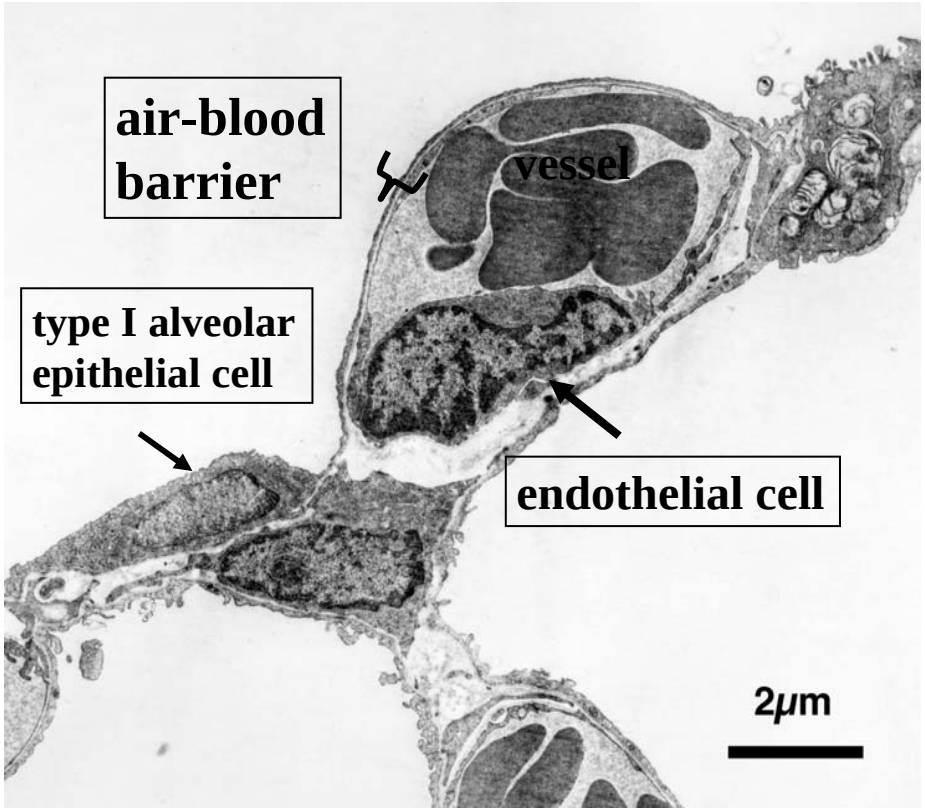
How are
Nanoparticles (<100nm)
different from conventional
particles (>100nm)
as to health effects?

Deposition of particles in the human respiratory system.

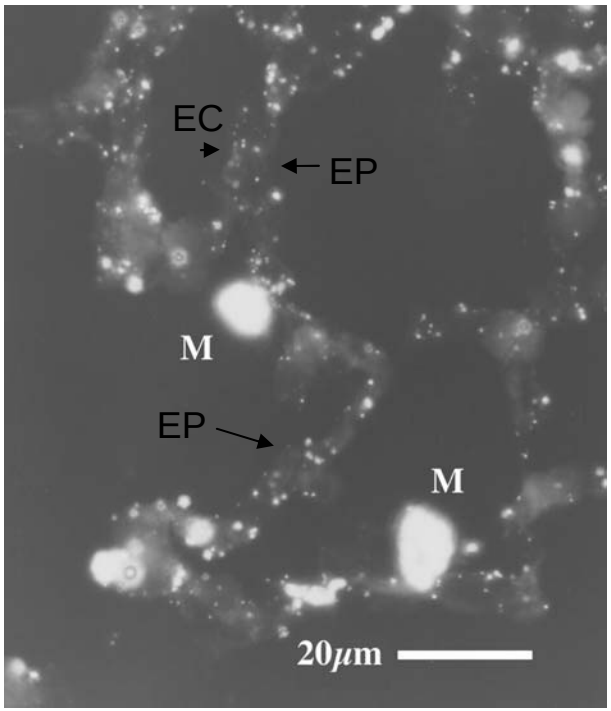


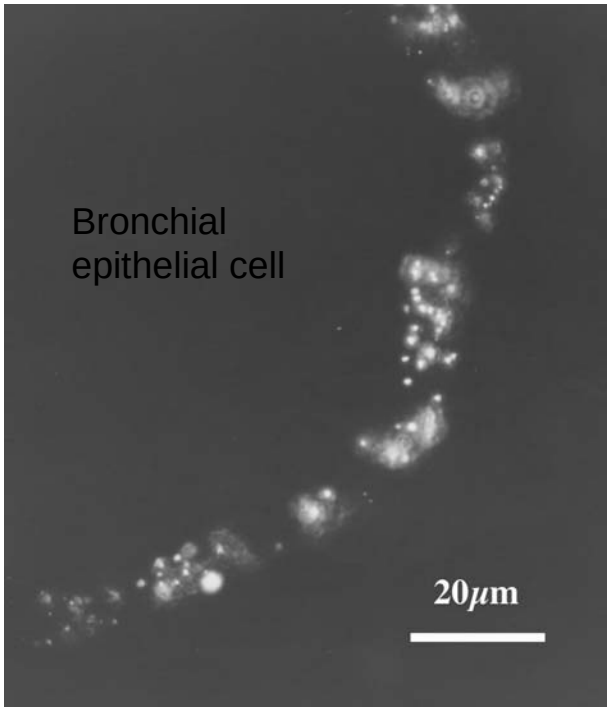
Structure of the alveolar region





Distribution of intratracheally instilled fluorescence-tagged polystyrene latex beads (24 nm) in the mouse lung.





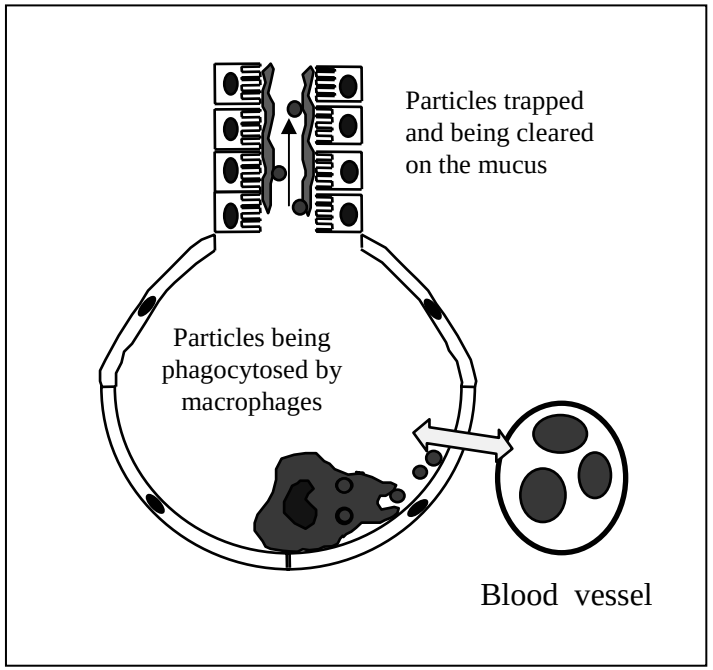
M: Macrophages
EP: Alveolar epithelial cells
EC: Vascular endothelial cells

by Furuyama, A.

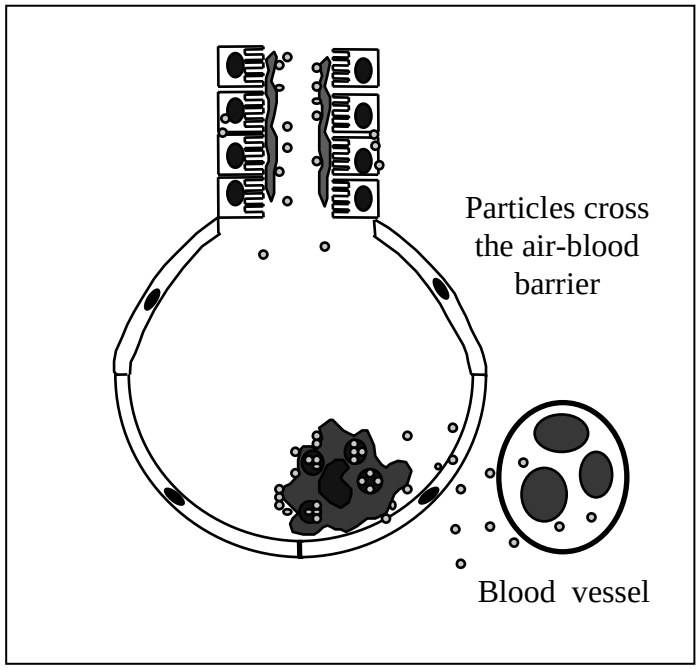
Small Particles - Big Problem

Stone V, and Donaldson K. (1998) Aerosol Soc. Newsletter, 33:12-14

PM10 / PM2.5



Ultrafine - Nano



Translocation of nanoparticles

Translocation through alveolar wall

- Pt: Oberdorster et al. (2000) 20nm 0.1mg/m^3 3h
- Ag: Takenaka et al. (2001) 15nm 0.1mg/m^3 3h
- Iridium: Kreyling et al. (2002) 20nm 0.1mg/m^3 1h
- ^{13}C-carbon: Oberdorster et al. (2002)
- Carbon graphite: Heyder (2003)

Translocation through olfactory cells

- ^{13}C-carbon: Oberdorster et al. (2004)

Health Effects of Manufactured nanoparticles

(1) Fullerenes

- What do we know about elemental carbon?

(2) Carbon nanotubes (SWCNT, MWCNT)

- What do we know about asbestos?
- Fibrous particles are more toxic than spherical ones?

(3) TiO_2, ZnO, CdSe

- What do we know about toxicology of heavy metal?

(4) DDS can be discussed separately

Fullerene

Buckminster Fuller

- Fullerene increased lipid peroxidation level in the brain and decreased glutathione level in the gill in fish.
  (E.Oberdorster, EHP, 112:1058, 2005)

- Inhibitor of potassium channel
  (K.H. Park, JBC, 278:50212, 2003)

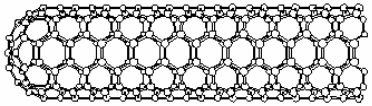
1 nm

- Water-soluble fullerenes accumulate in mitochondria
  (S.Foley, et al.,BBRC 294:116, 2002)

- Oxidative stressor after photoactivation
  (J.P. Kamat, et al.,Toxicology 155:55, 2000)

- Harmful in embryogenesis
  (Tsuchiya, T. et al., FEBS Lett., 393:139, 1996)

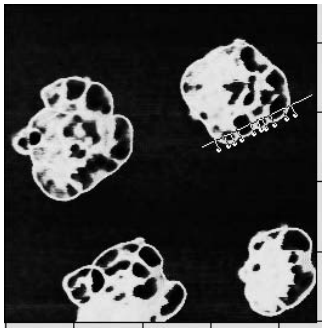
Carbon nanotubes



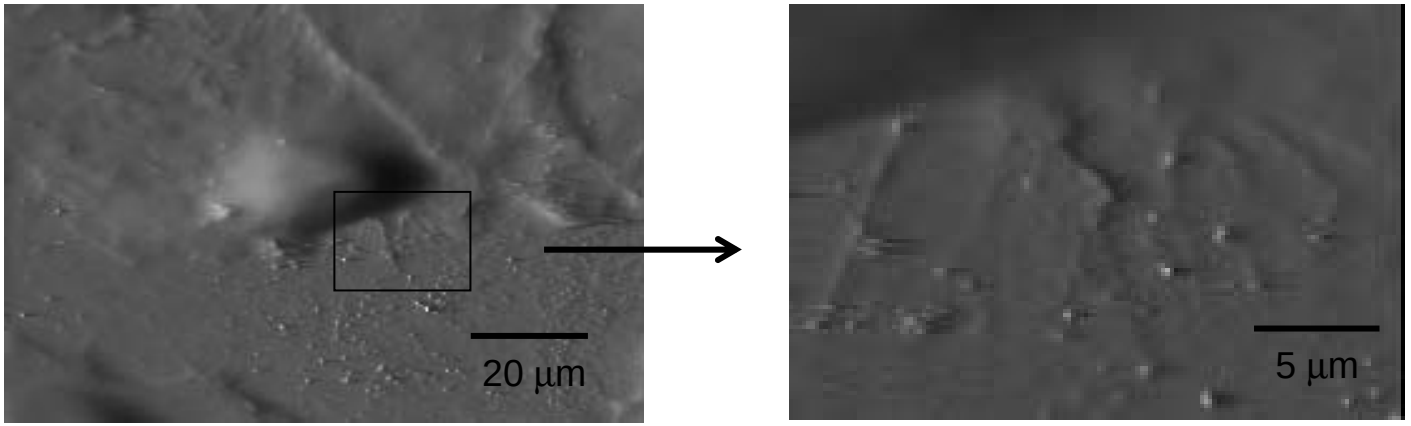
- Intratracheal instillation of CNT caused lung granulomas
  （C.-W. Lam, Toxicol.Sci.77:126, 2004)
  (D.B. Warheit, Toxicol Sci. 77:117, 2004)
- Inhibitor of potassium channel
  （K.H. Park, JBC, 278:50212, 2003)
- IL-8 production and cytotoxicity in keratinocytes
  （N.A. Monterio-Riviere,Toxicol. Lett 115:377, 2005)
- G_1 arrest and apoptosis in HEK239 cells
  （D.Cui, Toxicol. Lett, 155: 73, 2005)

Fullerene is a 100% carbon molecule and soluble in benzene

PVP (polyvinylpyrrolidone) or γ-cylodextrin can solubilize fullerene

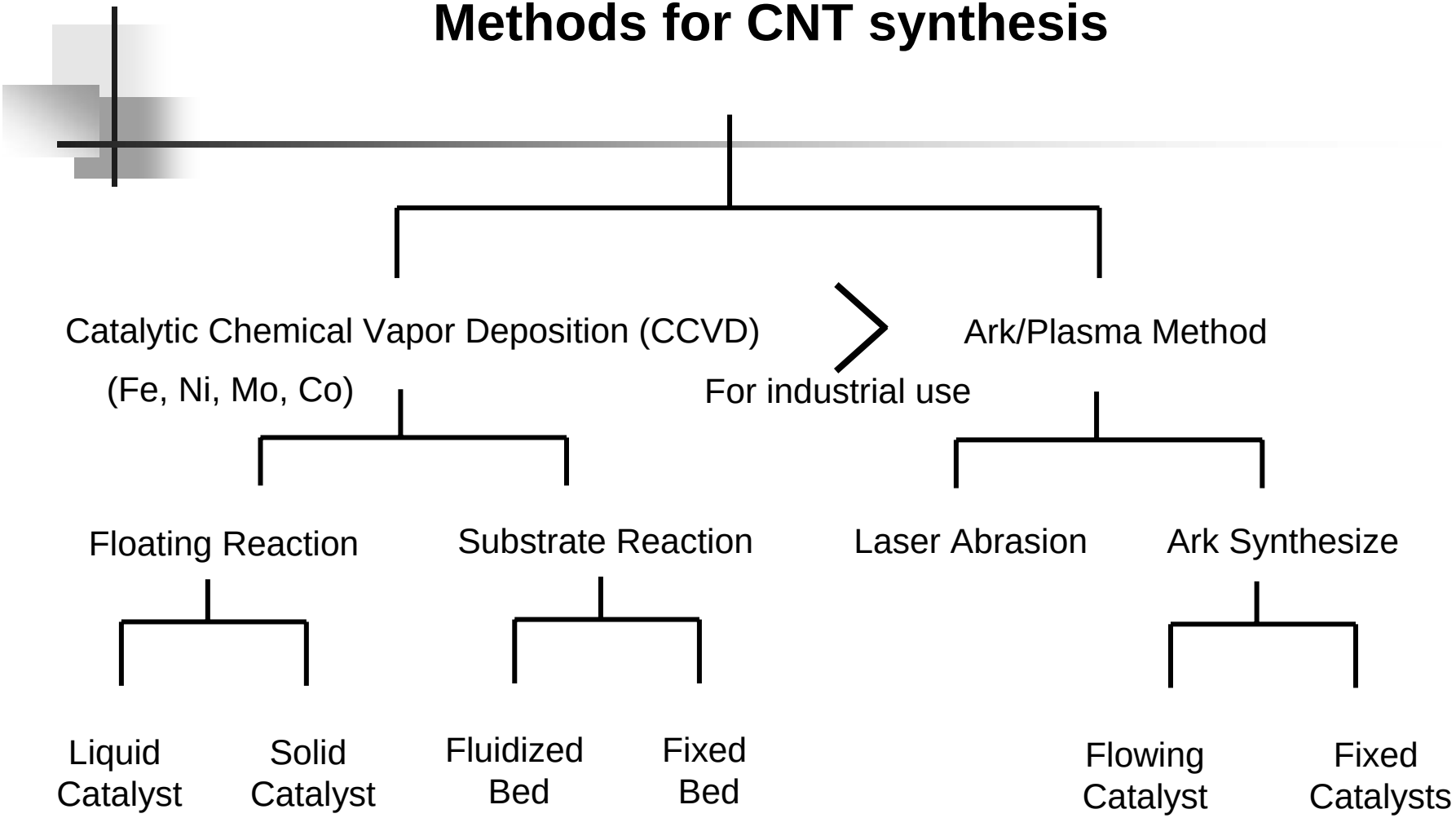


Fullerene on a poly-HEMA film



Endothelial cells phagocytoze crystalized fullerene particles (AFM)

Methods for CNT synthesis



Several reference samples are needed for safety evaluation of carbon nanotubes like UICC asbestos reference samples.

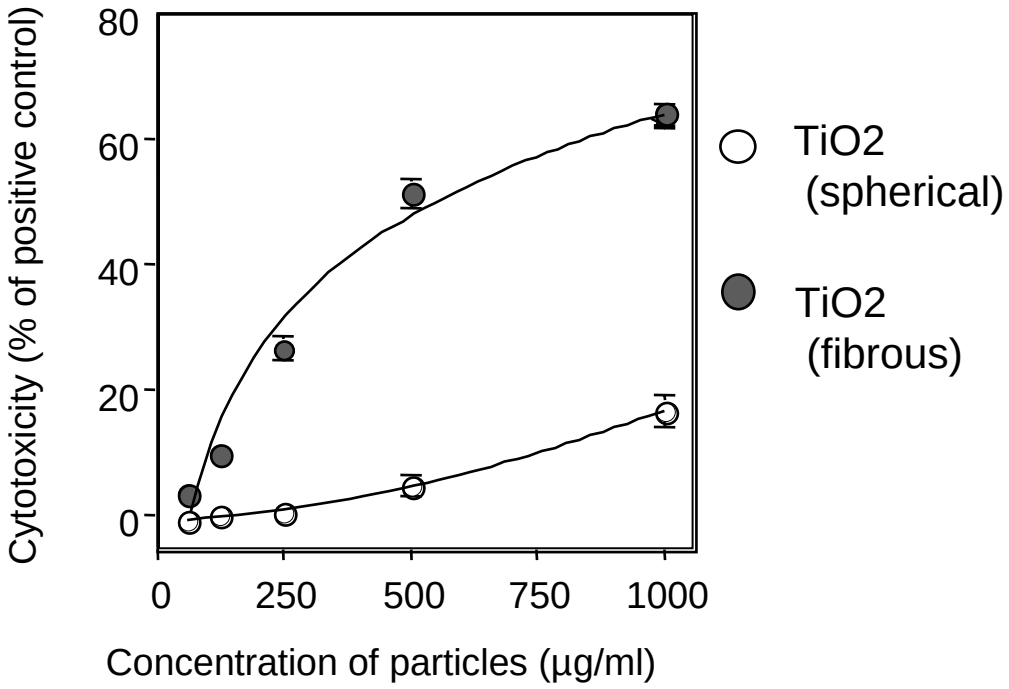
Why carbon now?

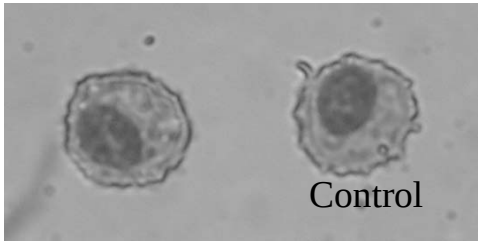
- Graphite
- Diamond
- Amorphous
- Fulleren
- Nanotube

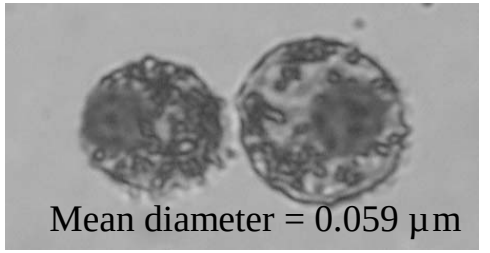
} Carbon Allotopes

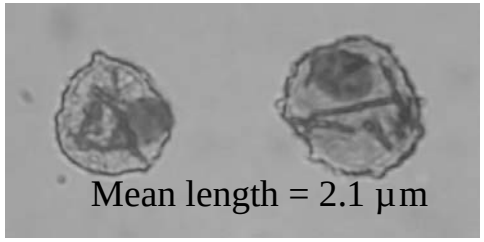
We used to recognize that carbons are inert/nuisance particles.

Cytotoxicity of Spherical and Fibrous TiO_2 in rat alveolar macrophages.



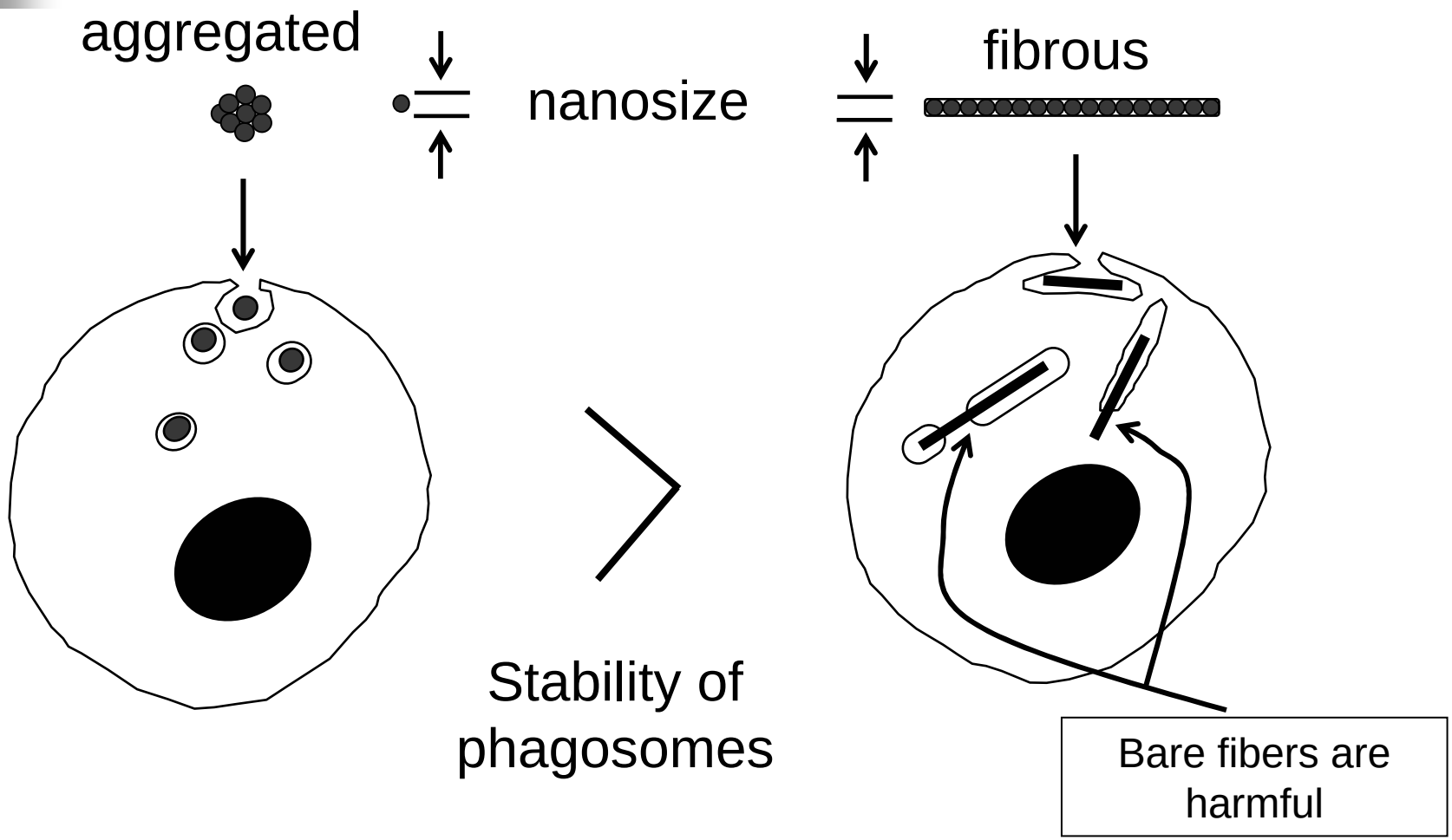






S.Hirano et al., Am. J. Respir. Cell Mol. Biol. 23:313–319 (2000)

Why are fibrous particles more cytotoxic ?



Amphibole asbestos-related lung diseases

Lippmann, M., Environ. Health Perspect., 88:311-317,1990

Esmen, N.A., Environ. Health Perspect., 88:277-286,1990

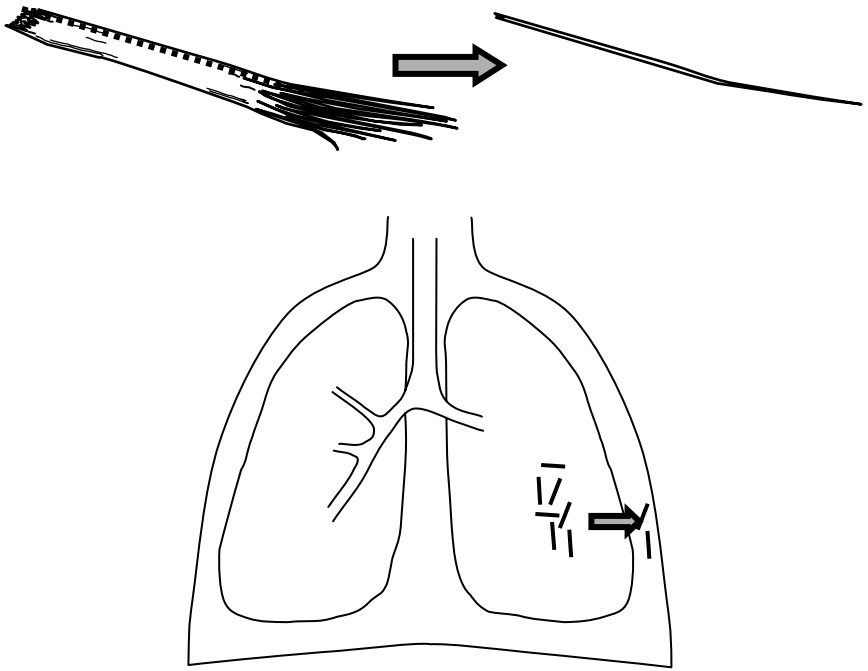
	Fiber length (μm)	Fiber width (μm)	Characteristic
Asbestosis	＞2	＞0.15	Biopersistent
Mesothelioma	＞5	＜0.1	Translocatable
Lung cancer	＞10	＞0.15	Biopersistent

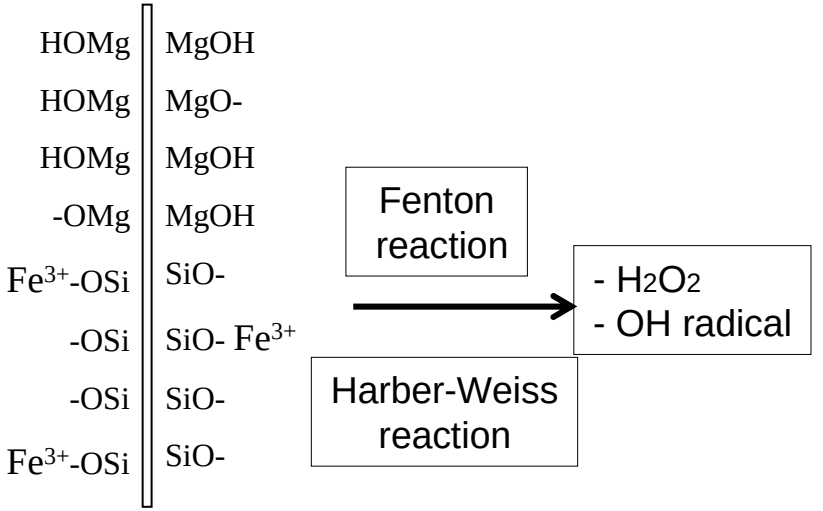
- Both biopersistence and permeability/translocatability are to be considered
- Thinner fibers move to pleura through lung tissue and cause mesothelioma
- Fibers with >100 μm length are not respirable
- Fibers with <2 μm length are fragments rather than harmful fibers in the lung.

Chrysotile asbestos fibers are found in mesothelioma

- Mt. Sinai asbestos research group.
- Ghio A.J., Toxicol Pathol 32:643-649 (2004)

Chrysotile asbestos cleave in the lung and generates translocatable fibers with a width of 15-40 nm (cf. 80-100 nm for amplibole)





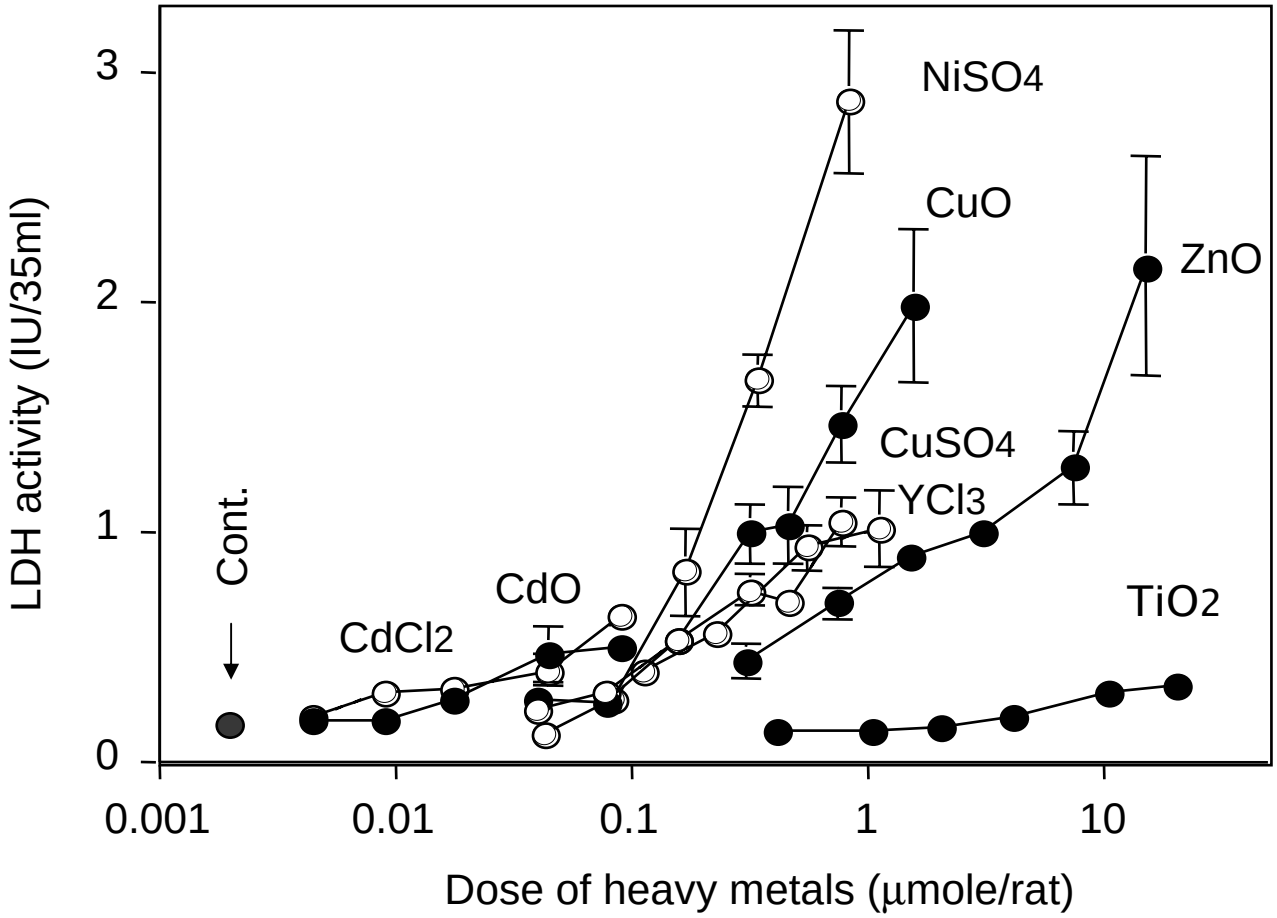
TiO_2 and other metal nanoparticles

Toxicity and health effects of heavy metals have been studies well.

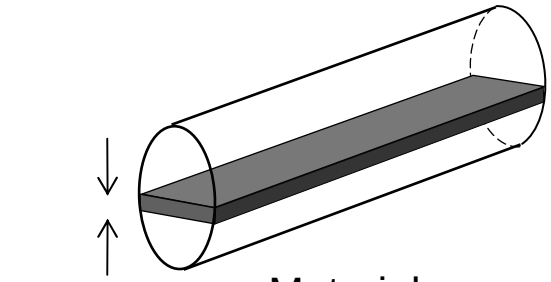
Do we really need a different strategy to evaluate metal nanoparticles?

Better to be considered, but is not necessarily prioritized.

Dose-dependent increase in LDH activity in bronchoalveolar lavage fluid following *i.t.* instillation of heavy metal compounds in rats. (S. Hirano and K.T. Suzuki (1996) Environ. Health Perspct.)



What are nanoparticles to be considered for safety evaluation?

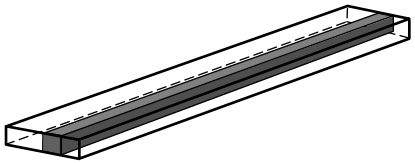


Nanosize

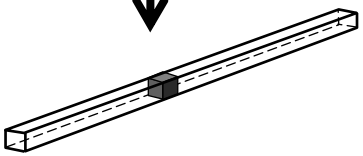
Material

1-100 nm thick

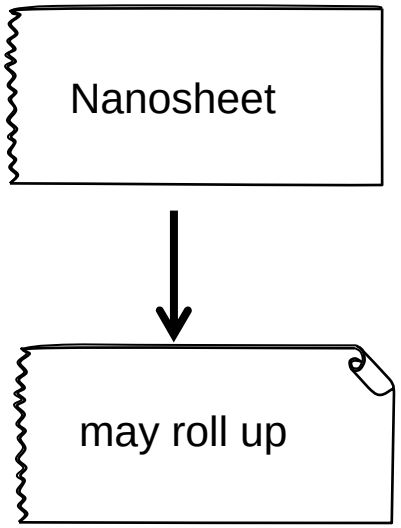
Nanoparticle

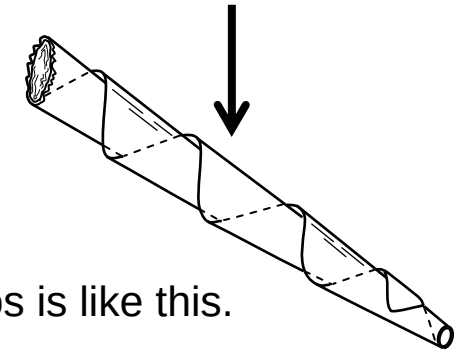


Nanosheet



Nanofiber
Nanowhisker
Nanorod





Chrysotile asbestos is like this.

Variables for Health Effects

Traditional health effects ＝ F(dose)

⇩

Nanotoxicity ＝ F（ mass, size, shape,
surface area and activity, etc）

and

<u>BIOPERSISTENCE</u>

Assay system for nanotoxicity

What is required to evaluate health-effect potency of manufactured nanoparticles?

-Inhalation studies are requisite	Intratracheal instillation of aggregates of nanoparticles may not reflect the toxicity of nanoparticles and adverse effects can be overestimated.
- Choose one of good metrics form size, shape, mass, surface area and activity, etc）	Surface area should be included. lung inflammation with TiO_2: 20 nm >> 250 nm (Oberdorster G. Environ Health Perspect. 102:173 (1994)
- Be aware of adsorbents	A total large surface is a good bed for toxicants such as endotoxin and organic materials.
-Chronic studies besides conventional assay	Biopersistence should be a key for most of manufactured nanoparticles

Robotics- or Biotech-risk can be applied for Nano-Risk

(Paradigm shift in risk perception/analysis)