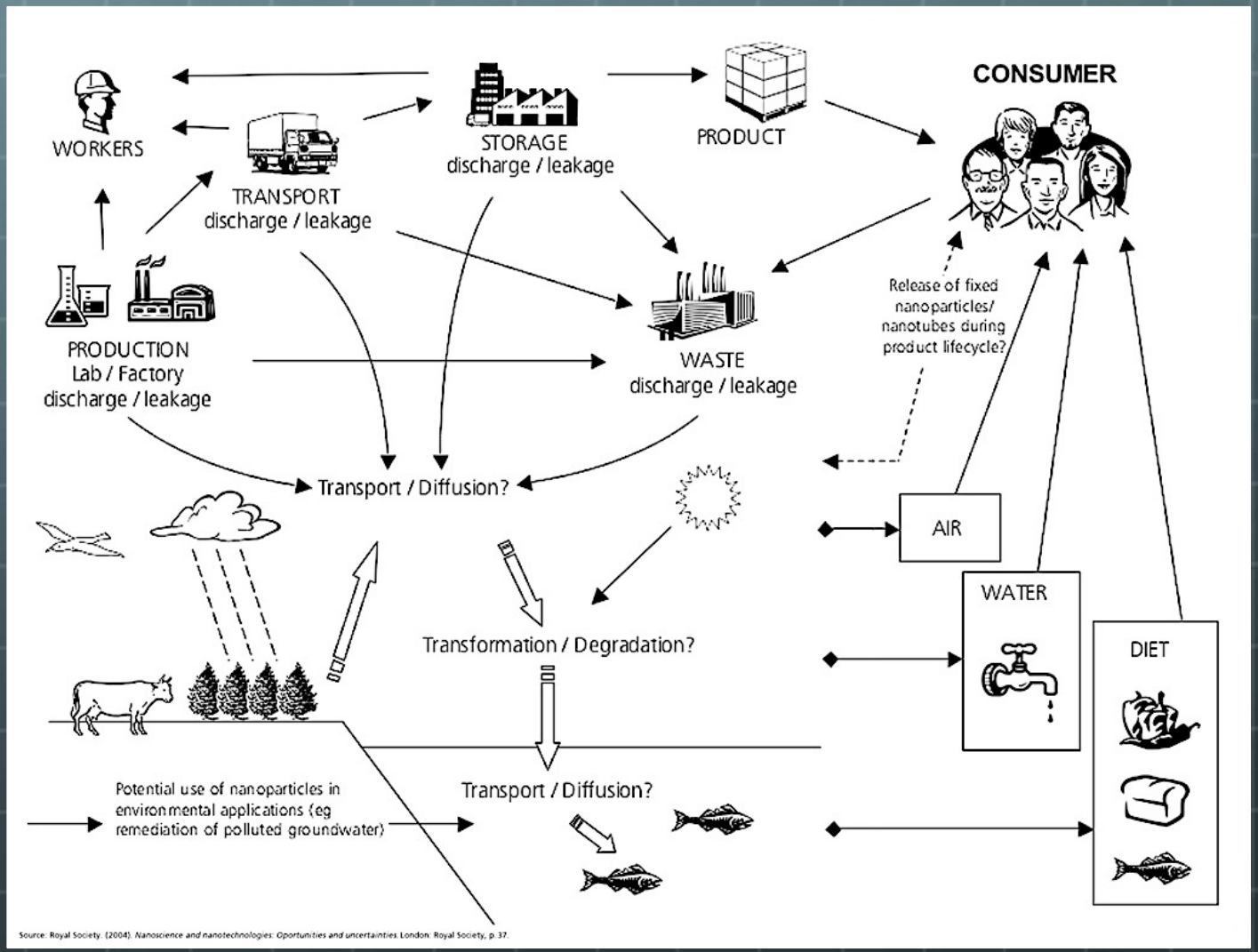
Influence of Particle Characteristics on Nanoparticle Toxicity: Size, Shape, Surface chemistry

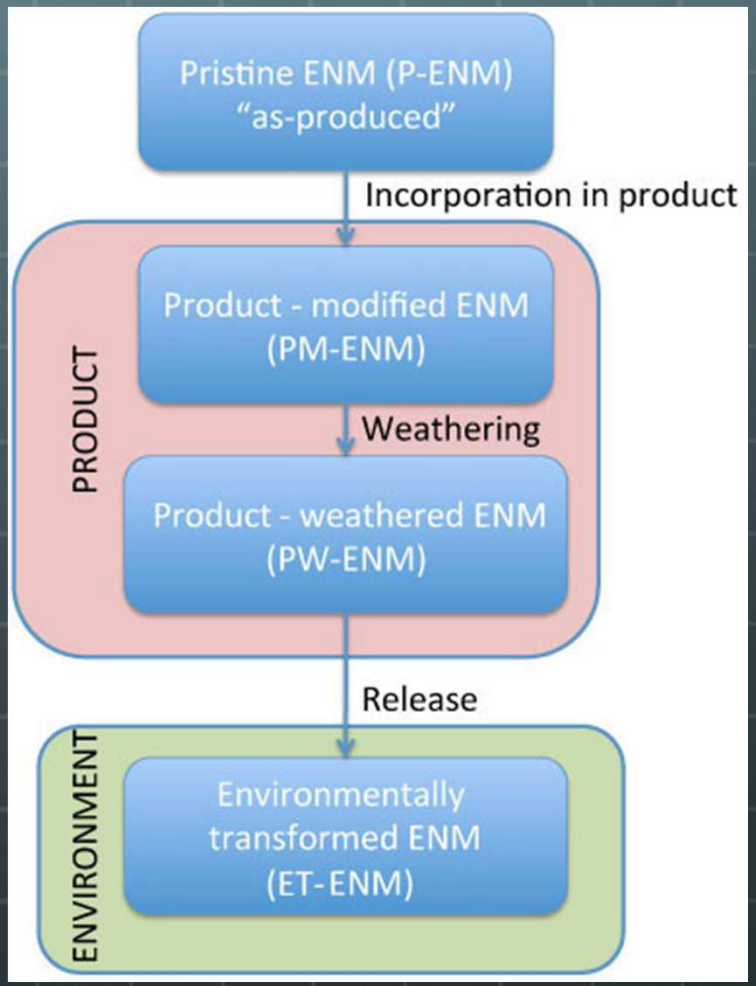
BIO1: Environmental Fate and Possible Effects of Nanoparticles
The 24th
Jyväskylä Summer School
Jyväskylän yliopisto

Routes to the Environment



Particle Transformations

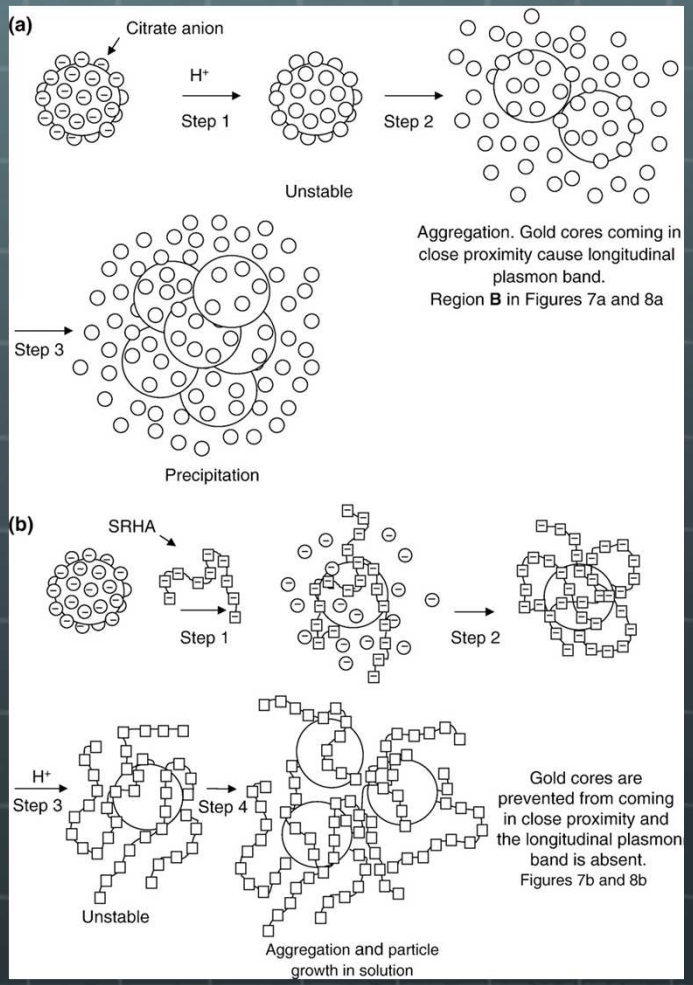
- Nanomaterials are designed for a specific purpose though manipulation of the physicochemical properties
- These particle properties are highly susceptible to change throughout the "life" of the nanomaterial
- Examples of particle transformations :
  - Shielding ROS production in TiO_2 sunscreens via Al/Si coatings
  - Coating of nanomaterial by organic components in wastewater
  - Coating of nanomaterial by natural organic matter in aqueous systems



Nowack et al. 2012

Particle Behavior

- A function of:
  - The intrinsic and adopted particle characteristics
    - Size
    - Shape/Aspect ratio
    - Surface charge/chemistry
    - Surface area
  - Water Chemistry/Water Quality
    - pH
    - Organic matter content
    - Ionic strength
    - Redox environment
    - Temperature
  - Abiotic Processes
    - Seasonal Mixing
    - Chemical Complexation/ Oxidation-Reduction Rxn
    - UV-Light --> photo-activation



Diegoli et al. 2008

Bioavailability

- Bioavailability a function of the partitioning between environmental compartments
- Interactions with the biota can impact partitioning
  - excretion of nanomaterials in solid waste introduces suspended nanomaterials to benthic organisms
  - Disturbance of settled nanomaterials in sediments may reintroduce them to pelagic organisms
- Adopted and intrinsic particle characteristics important to partitioning

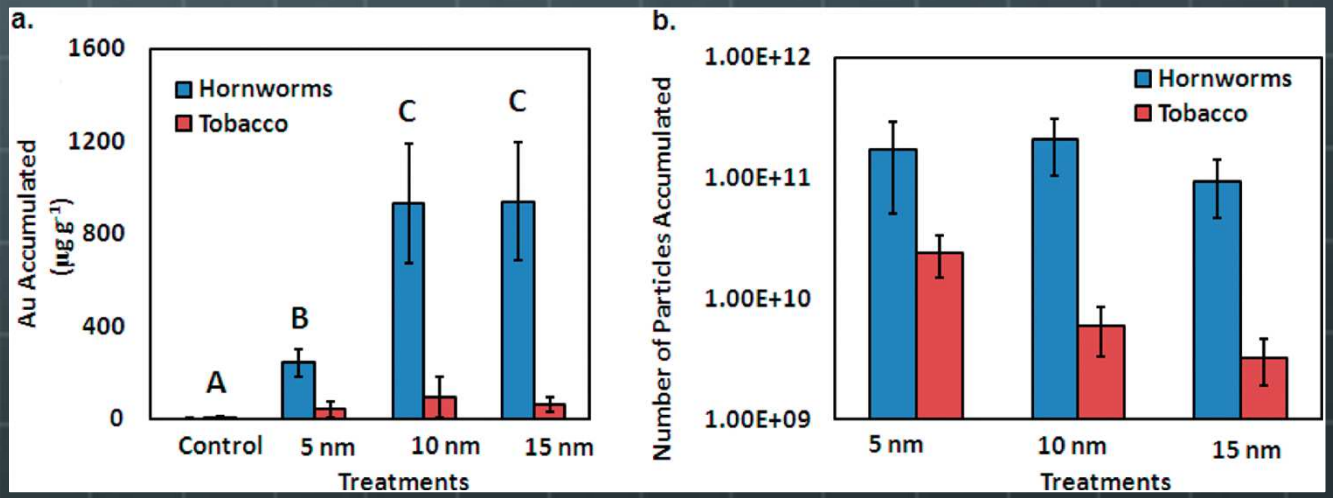
Table 2. Ratiometric Comparison of Negatively and Positively Charge-Stabilized Particle Fates

phase	C_{f-}/C_{f+}	(percentage of negatively charged nanopartides recovered)/(percentage of positively charged nanoparticles recovered)[a]
sea water	1.00	0.17
sediment	30.9	3.18
biofilm	18.9	0.32
S. alterniflora	2.53×10^2	3.30
P. pugio	67.0	4.00
I. obsoleta	11.3	0.20
M. mercenaria	0.15	0.02

Burns et al. 2013

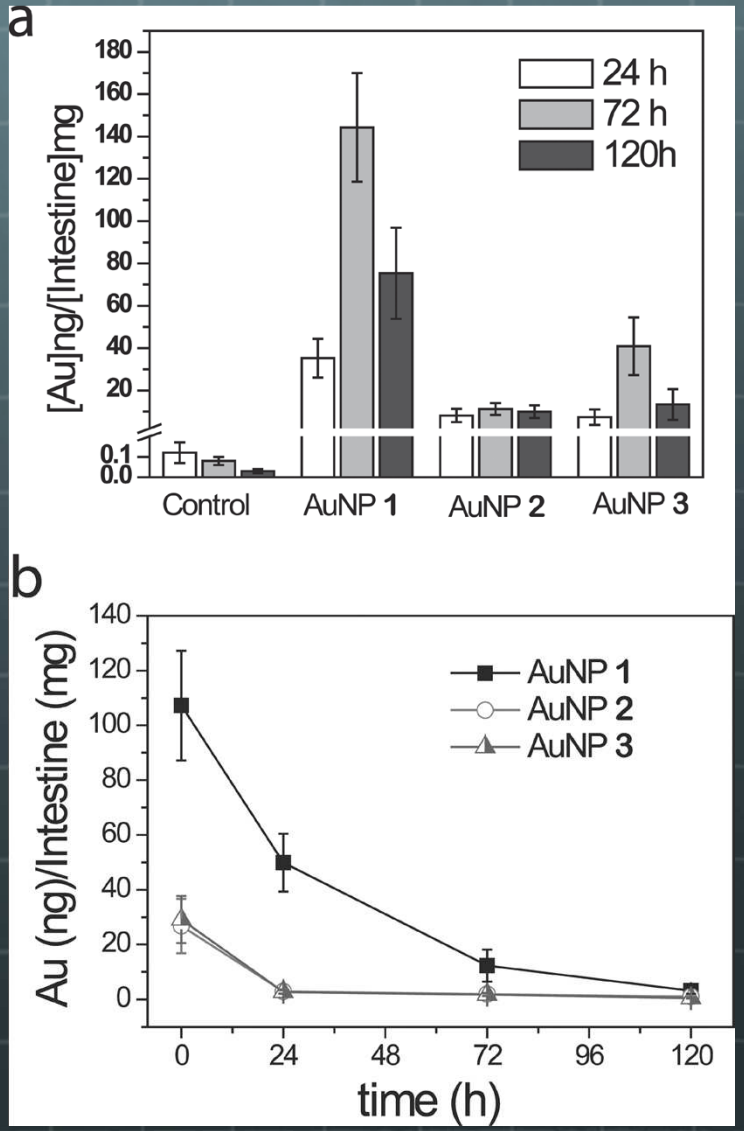
Accumulation

- Bioconcentration of nanomaterials varies based on particle characteristics and organism physiology (Wray and Glenn work from yesterday)
- Dietborne accumulation is an important route for certain species
  - Trophic transfer does occur
  - Biomagnification rare (Werlin et al. 2010, Judy et al. 2011)



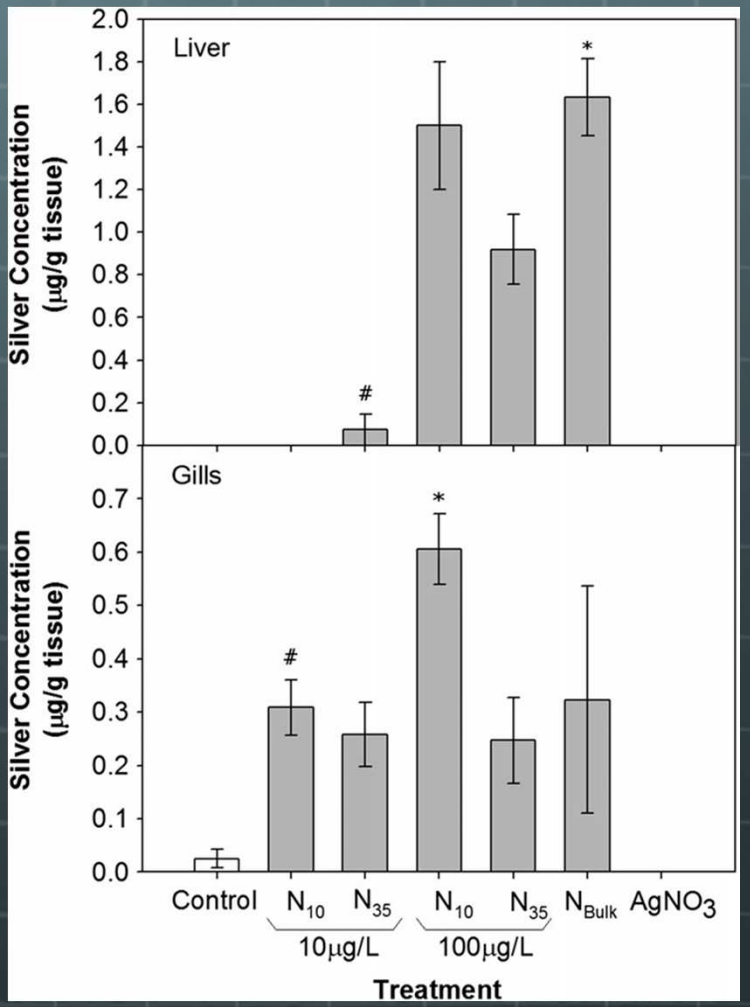
Accumulation

- Zhu et al. 2010 – particle surface charge/chemistry affects uptake (a) and elimination (b)



Distribution

- Internal distribution can be a function of particle characteristics
- Scown et al. 2010 - Rainbow trout accumulation in the gills and liver is size dependent



Scown et al. 2010

Distribution

- Zhu et al. 2010– particle distribution based on surface charge

Table 1. Biodistribution of AuNPs **1–4** in fish organs at 24, 72, and 120 h.

Organ	Control [d]	AuNP **1** [d]	AuNP **2** [d]	AuNP **3** [d]	AuNP **4** [d]
	Au [ng/mg]	Au [ng/mg]	Au [ng/mg]	Au [ng/mg]	Au [ng/mg]
Brain [a]	0.35 ± 0.14	1.10 ± 0.49	0.27 ± 0.08	0.20 ± 0.04	1.29 ± 0.15 ***
Heart [a]	2.28 ± 1.11	19.0 ± 9.59	5.03 ± 3.68	1.57 ± 0.77	16.0 ± 5.3 *
Liver [a]	0.19 ± 0.09	1.12 ± 0.57	0.22 ± 0.09	0.18 ± 0.04	0.99 ± 0.23 *
Gonad [a]	0.13 ± 0.05	12.1 ± 11.1	0.18 ± 0.08	0.09 ± 0.03	6.05 ± 3.97
Dorsal fin [a]	1.35 ± 0.76	1.03 ± 0.19	3.41 ± 2.69	0.76 ± 0.32	14.3 ± 3.4 **
Gill [a]	0.24 ± 0.09	5.07 ± 1.38 **	0.53 ± 0.11	0.16 ± 0.03	25.5 ± 5.7 ***
Intestine [a]	0.12 ± 0.05	35.2 ± 9.1 **	8.15 ± 3.19 *	7.35 ± 3.68	0.86 ± 0.29 *
Gill [b]	0.25 ± 0.12	2.17 ± 0.42 ***	0.63 ± 0.08 **	0.33 ± 0.09	—
Intestine [b]	0.08 ± 0.02	144 ± 26 ***	11.2 ± 2.8 **	40.9 ± 13.7 **	—
Gill [c]	0.10 ± 0.04	1.09 ± 0.08 ***	0.48 ± 0.09 **	0.24 ± 0.03 *	—
Intestine [c]	0.03 ± 0.01	75.4 ± 21.6 *	9.95 ± 3.00 *	13.3 ± 7.3	—

[a] 24 h, [b] 72 h, and [c] 120 h. [d] The concentration unit refers to ng of Au per mg of organ weight; Means are averaged from eight fish (24 h and 72 h) or four fish (120 h) and accompanied by standard error of the mean (SEM); For AuNP **4**, all of the fish died in 24 h, no data available for 72 and 120 h. * P≤ 0.05, **P≤ 0.01, ***P≤ 0.001 through one-way ANOVA between control and AuNP treated groups, see detail p values in Tables S1–4 in Supporting Information.

Ecotoxicology of Nanomaterials

- It should come as no surprise that lethal and sub-lethal toxicity is dependent on particle characteristics, water chemistry, and organism physiology
  - Toxicity varies based on preparation methods (dispersants, excess ligand concentration, etc.)
- In many cases the nanomateiral LC50 is far in excess of the predicted or measured environmental concentration (Gottschalk et al. 2009)
  - Exceptions – AgNPs, ZnO, and TiO_2

Toxic Mechanisms

- Ion release
  - Certain nanomaterials (Ag, Zn, Cu) are highly susceptible to oxidation and dissolution in aqueous media
  - The smaller the nanomaterial the greater the dissolution rate
  - Identifying the cause of toxicity for nanomaterials that readily dissolve has proven difficult
    - Studies have shown nanospecific toxicity that differs in response and degree from known ion toxicity (Shaw et al. 2012, Chae et al. 2009)
    - Other studies using similar particles have linked toxicity strictly to ionic release (Kim et al. 2012)
- Generation of reactive oxygen species
  - Common for metallic and metal oxide nanomaterials
  - Leads to oxidative stress, depletion of glutathione, lipid peroxidation, cell damage and death
- Energetics
  - Nanomaterials can coat and clog the intestinal tract of an organisms inhibiting absorption of nutrients and requiring more energy to excrete (Zhu et al. 2011)
  - Important in filter feeding species such as *Daphnia*
- Carbon nanotubes = asbestos?
- Trojan horse
  - Nanomaterials may increase bioavailability of other contaminants

Surface Chemistry

- Uncoated versus polysaccharide coated Ag NP
  - Uncoated tended to agglomerate more
  - Uptake was studied in 2 cell lines
    - Both were taken up
    - Uncoated were agglomerated and stayed in cytoplasm
    - Coated were evenly distributed throughout cell
  - Coated seemed to cause more toxicity
    - DNA damage
    - Up regulation of proteins
    - Apoptosis

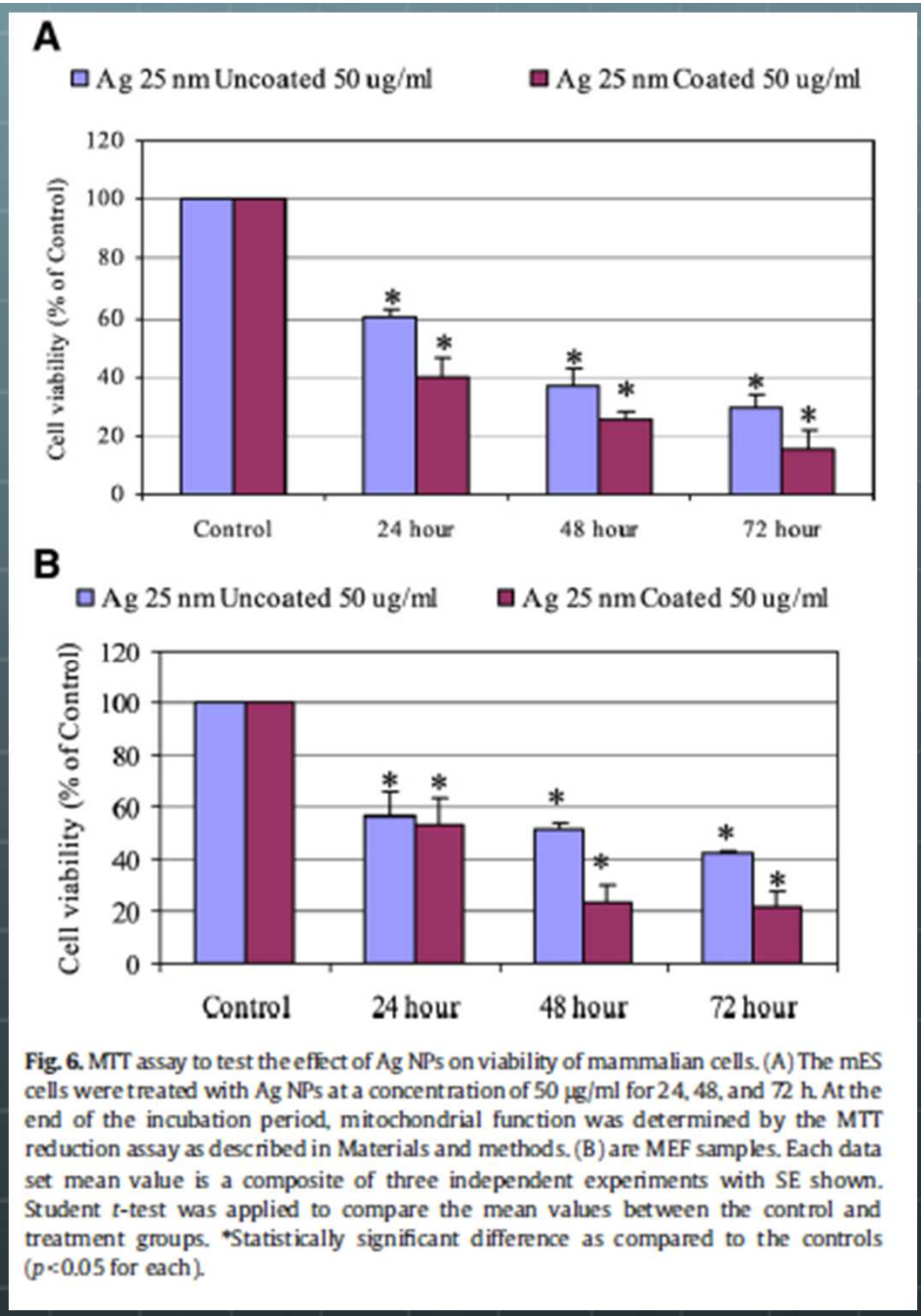


Fig. 6. MTT assay to test the effect of Ag NPs on viability of mammalian cells. (A) The mES cells were treated with Ag NPs at a concentration of 50 μg/ml for 24, 48, and 72 h. At the end of the incubation period, mitochondrial function was determined by the MTT reduction assay as described in Materials and methods. (B) are MEF samples. Each data set mean value is a composite of three independent experiments with SE shown. Student *t*-test was applied to compare the mean values between the control and treatment groups. *Statistically significant difference as compared to the controls ($p<0.05$ for each).

Ahmed et al 2008

Surface Charge

- Goodman et al. 2004 – MTT assay for cell viability reveals cationic surface charge to be more toxic

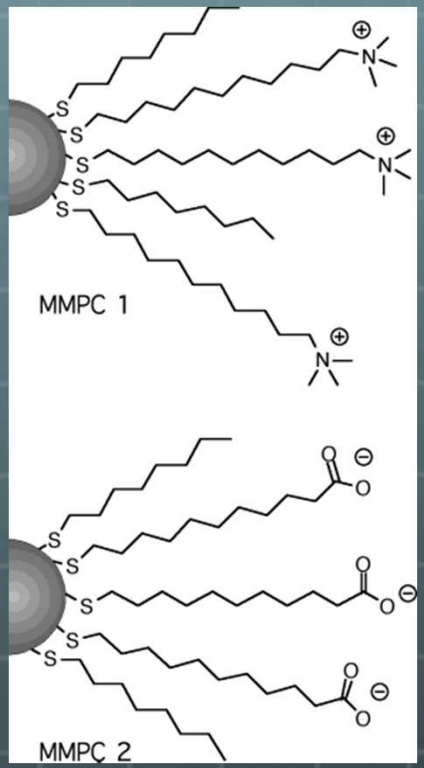


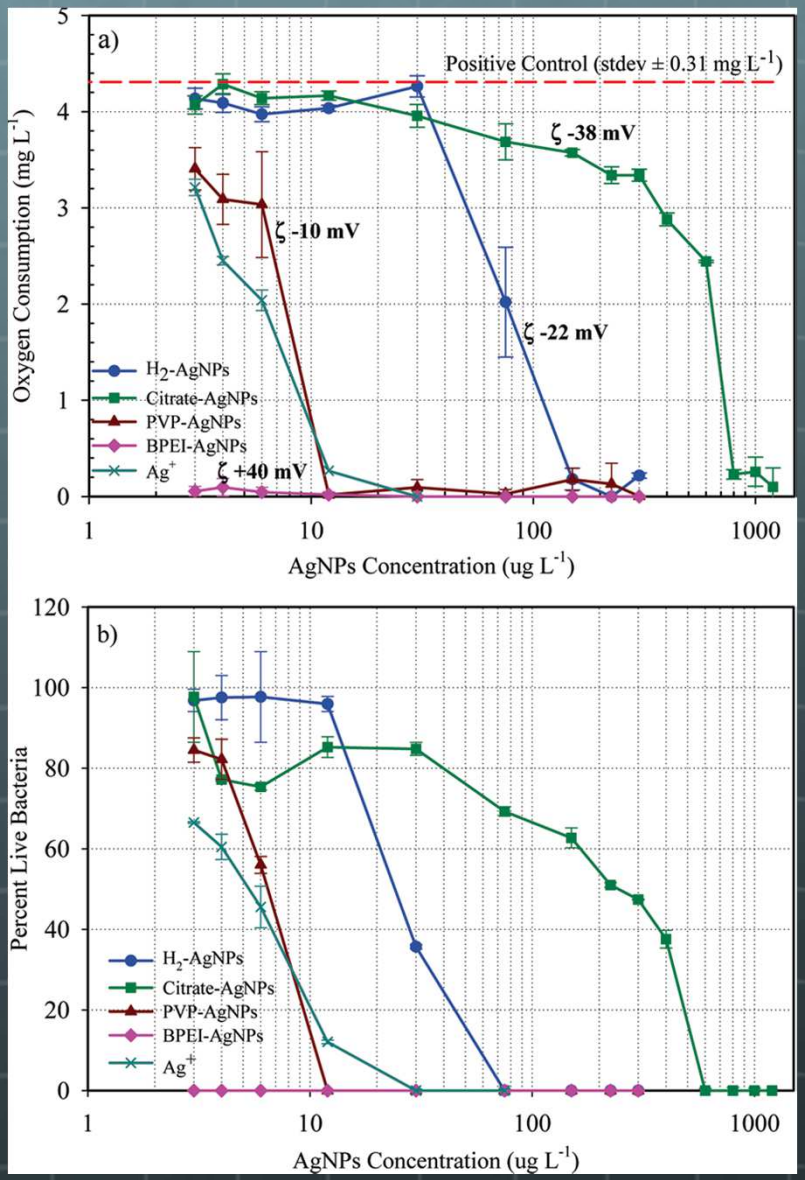
Table 1. LC_{50} Values of MMPC 1 and 2 in Mammalian Cells and *E. coli*

	MMPC **1**, μM	MMPC **2**, μM
Cos-1	1.0 ± 0.5[a]	>7.37[b]
red blood cells	1.1 ± 0.1	72 ± 18
E. coli	3.1 ± 0.6	>28[c]

[a] LC_{50} value for MMPC **1** observed after 1 h of nanoparticle incubation. [b] Cells were 100% viable after 24 h of incubation with MMPC **2**. Higher concentrations of nanoparticles could not be completely washed from wells, and interfered with absorbance readings. [c] Higher concentrations could not be tested due to decreasing visibility of the colonies on the nanoparticle-doped agar.

Surface Charge

- Badawy et al. 2011 – toxicity as measured by oxygen consumption and percent live bacteria reveal differences based on surface charge
- Cationic nanomaterial (BPEI) is the most toxic, anionic nanomaterial (Citrate) least toxic
- Repulsion between anionic particles and cellular surface is likely cause of reduced toxicity



Badawy et al. 2011

Surface Charge/Chemistry

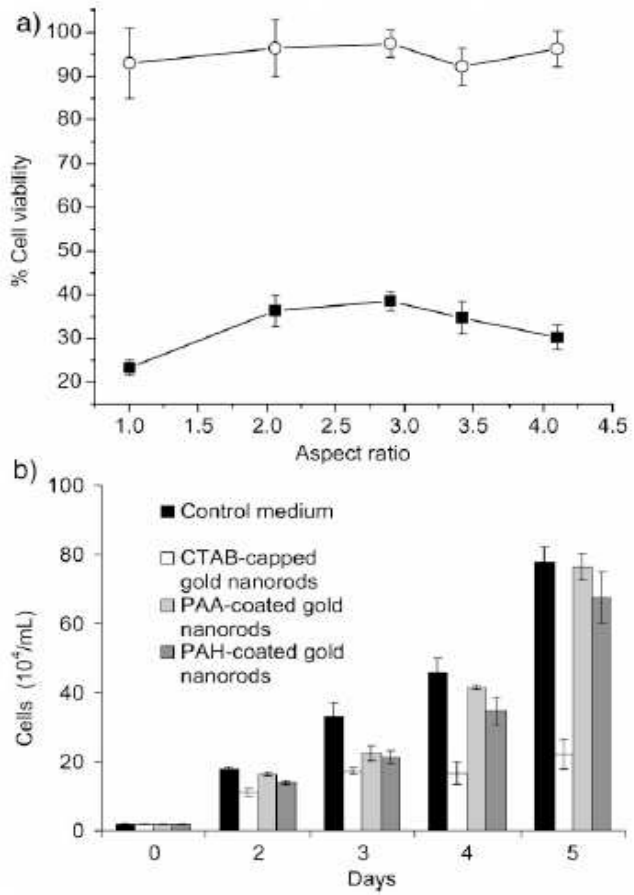


Figure 2. a) Viability of HT-29 cells exposed to 0.4 nM of either CTAB-capped gold nanorod solutions (■) or PAA-coated gold nanorod solutions (○) for four days as a function of gold nanorod aspect ratio. b) Growth of HT-29 cells exposed to 0.4 nM of CTAB-capped gold nanorod solutions for five days. Error bars represent one standard deviation.

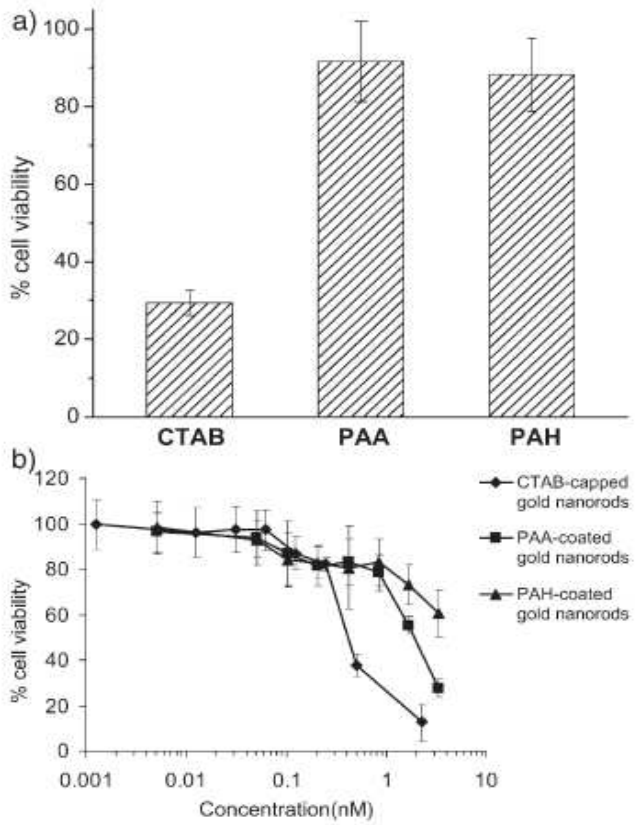


Figure 3. Viability of cells exposed to gold nanorods with different surface coatings. a) Viability of HT-29 cells exposed to 0.4 nM of CTAB-, PAA- and PAH-coated gold nanorod solutions for four days. Aspect ratios of all gold nanorods were 4.1. b) Dose-dependent viability of HT-29 cells exposed to increasing concentrations of CTAB-, PAA-, and PAH-coated gold nanorod solutions (aspect ratios of 4.1). Error bars represent one standard deviation.

Alkilany et al. 2009

Size

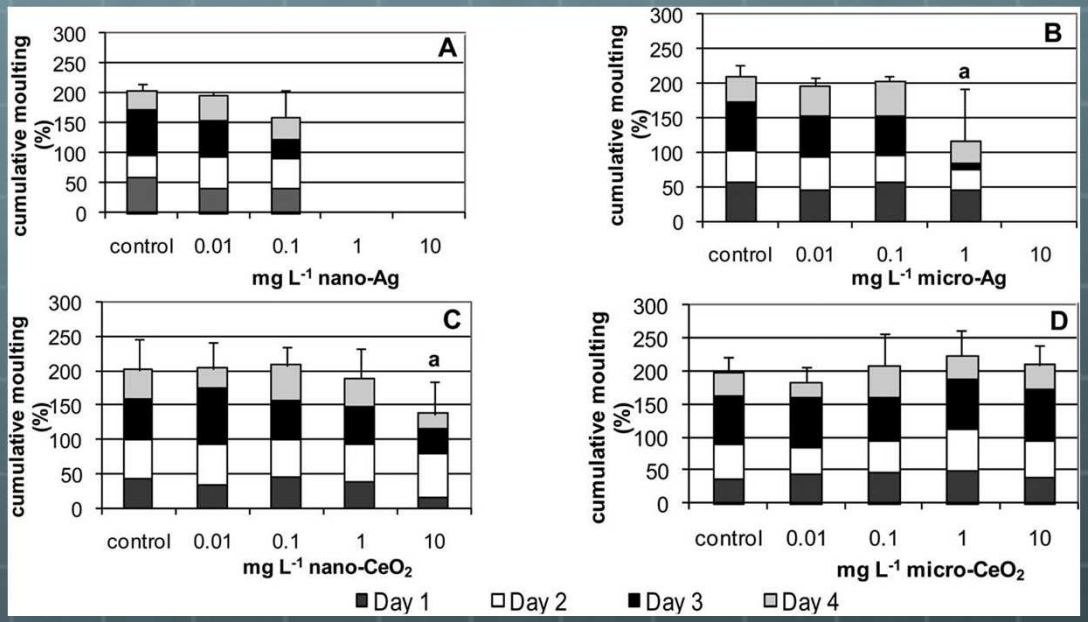
Table 1 Acute toxicity of particles to *D. magna*. Neonates were exposed to particles for 96 h. Three (Ag) or four (CeO_2) experiments were performed with $n = 10$ replicates per treatment in each experiment. Figures represent mean survival rates ± standard error of the mean

Particle Type	0 (control)	0.01 mg L^{-1}	0.1 mg L^{-1}	1 mg L^{-1}	10 mg L^{-1}
nano-Ag	100 ± 0	93.33 ± 3.33	43.33 ± 23.33[a]	0 ± 0[c]	0 ± 0[c]
micro-Ag	100 ± 0	96.67 ± 3.33	β6.67 ± 6.67	20 ± 20[b]	0 ± 0[c]
nano-CeO_2	94.59 ± 3.13	94.72 ± 3.06	97.5 ± 2.5	86.88 ± 2.37	92.5 ± 4.79
micro-CeO_2	94.59 ± 3.13	97.22 ± 2.78	95 ± 2.89	95 ± 2.89	95 ± 2.89

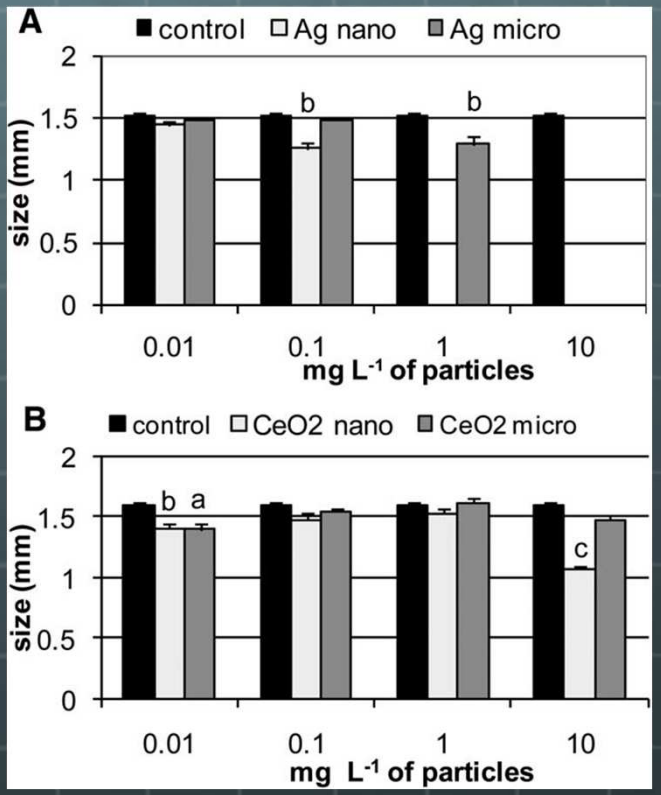
[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$ compared to untreated control.

Gaiser et al. 2011

Size



Cumulative molting of *D. magna* during 96 hr test



D. magna neonate size after 96 hr test

Gaiser et al. 2011

Size

- Au and Ag Nanoparticles
  - 3 sizes (s 2-4 nm; m, 5-7 nm; L, 20-40 nm)
  - Treated macrophages
  - Characterized nanoparticles
  - Cytotoxicity

Table 1. The zeta potential of AuNPs and AgNPs of different sizes.

	Size [nm]	Zeta potential [mV]
Au-S	2.81 ± 0.84	−56.64 ± 1.84
Au-M	5.52 ± 0.95	−60.85 ± 2.88
Au-L	38.05 ± 11.88	−78.81 ± 1.97
Ag-S	3.08 ± 1.16	13.69 ± 0.25
Ag-M	5.75 ± 1.12	15.43 ± 2.72
Ag-L	24.85 ± 6.06	5.35 ± 1.26

Yen et al 2009

Size

Smaller sizes reduced number of cells

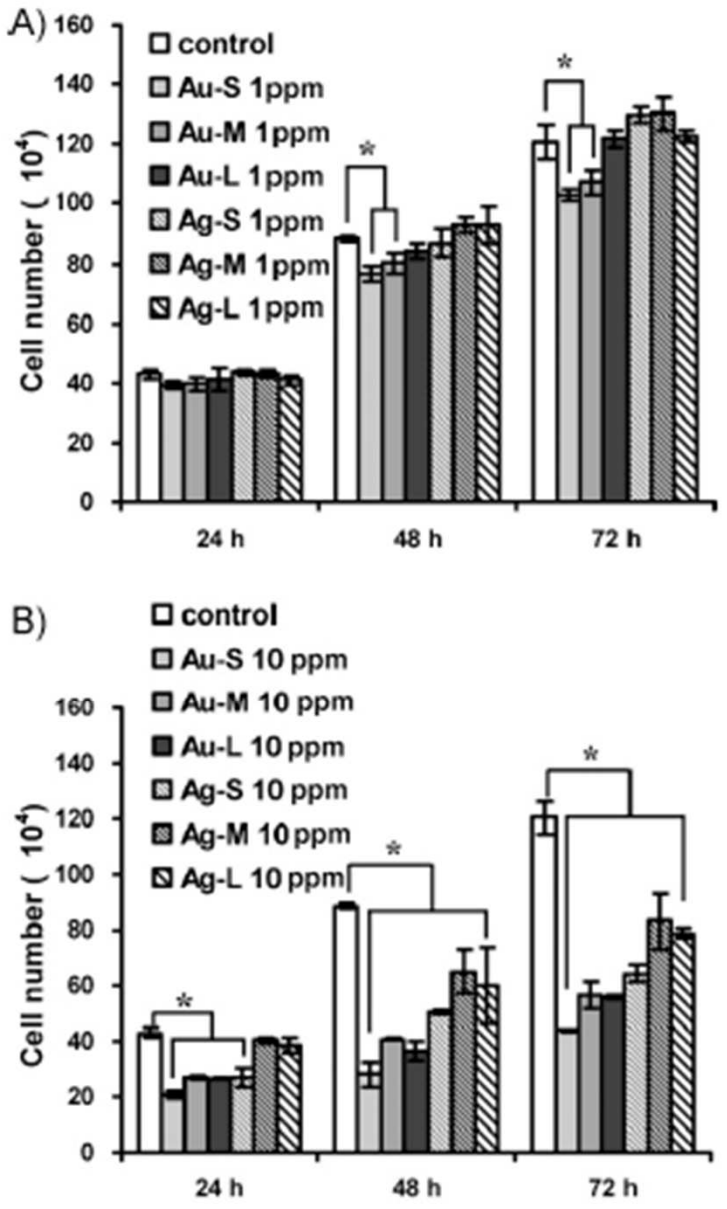


Figure 4. Cytotoxicity of AuNPs and AgNPs on J774 A1 macrophages. The cells were incubated with culture medium containing NPs (of different size) at concentrations of 1 ppm (A) and 10 ppm (B) for 24, 48, and 72 h. Significance $p < 0.05$: * less than control.

Size

Round Shape

Spread Shape

Figure 5. A) The original morphology of J774 A1 macrophages. B) Cell morphology after treatment with different sizes of AuNPs or AgNPs at 1 ppm for 24 h. C) Cell morphology of macrophages after treatment with different sizes of AuNPs or AgNPs at 10 ppm for 24 h. The formation of many vesicles was observed in cells treated with AuNPs at 10 ppm. The cellular uptake of NPs was clearly observed in cells treated with either type of NP at 10 ppm.

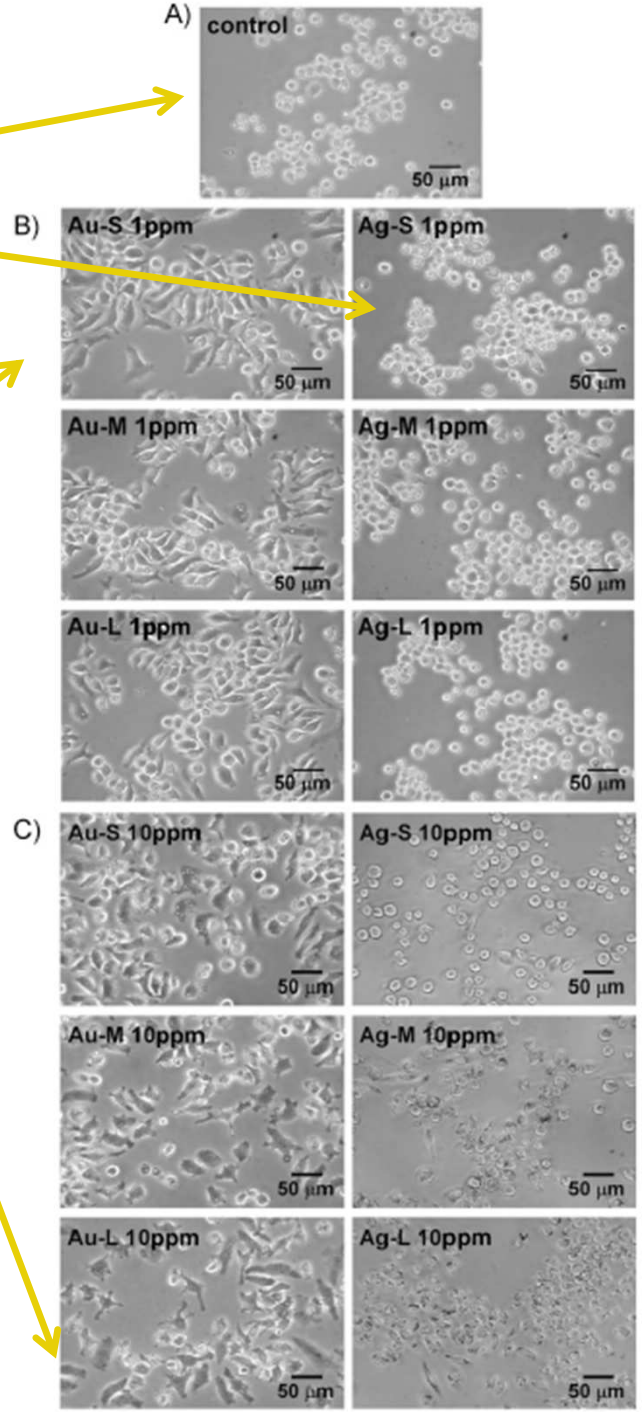


Table 2. The average size of J774 A1 macrophages treated with AuNPs or AgNPs of various sizes. The cells were incubated with culture medium containing NPs at 1 ppm and 10 ppm for 24, 48, and 72 h. Significance $p < 0.05$: * cell average size larger than control.

Specimens	Cell average size [μm]		
	24 h	48 h	72 h
control	15.52 ± 0.09	15.40 ± 0.29	15.27 ± 0.41
Au-S 1 ppm	17.08 ± 0.14*	16.82 ± 0.34*	16.05 ± 0.30*
Au-M 1 ppm	17.23 ± 0.08*	16.70 ± 0.33*	15.84 ± 0.08*
Au-L 1 ppm	16.93 ± 0.13*	16.33 ± 0.34*	15.34 ± 0.16
Au-S 10 ppm	18.18 ± 0.02*	18.18 ± 0.09*	18.26 ± 0.10*
Au-M 10 ppm	17.87 ± 0.30*	17.08 ± 0.12*	17.70 ± 0.29*
Au-L 10 ppm	17.75 ± 0.02*	16.96 ± 0.18*	17.24 ± 0.07*
Ag-S 1 ppm	15.43 ± 0.09	14.84 ± 0.23	14.43 ± 0.40
Ag-M 1 ppm	15.46 ± 0.08	15.16 ± 0.18	14.84 ± 0.33
Ag-L 1 ppm	16.02 ± 0.11*	15.50 ± 0.29	15.04 ± 0.27
Ag-S 10 ppm	16.64 ± 0.11*	15.96 ± 0.21*	15.39 ± 0.21
Ag-M 10 ppm	16.12 ± 0.14*	15.67 ± 0.40	15.13 ± 0.27
Ag-L 10 ppm	16.46 ± 0.04*	16.26 ± 0.24*	15.81 ± 0.30

Size

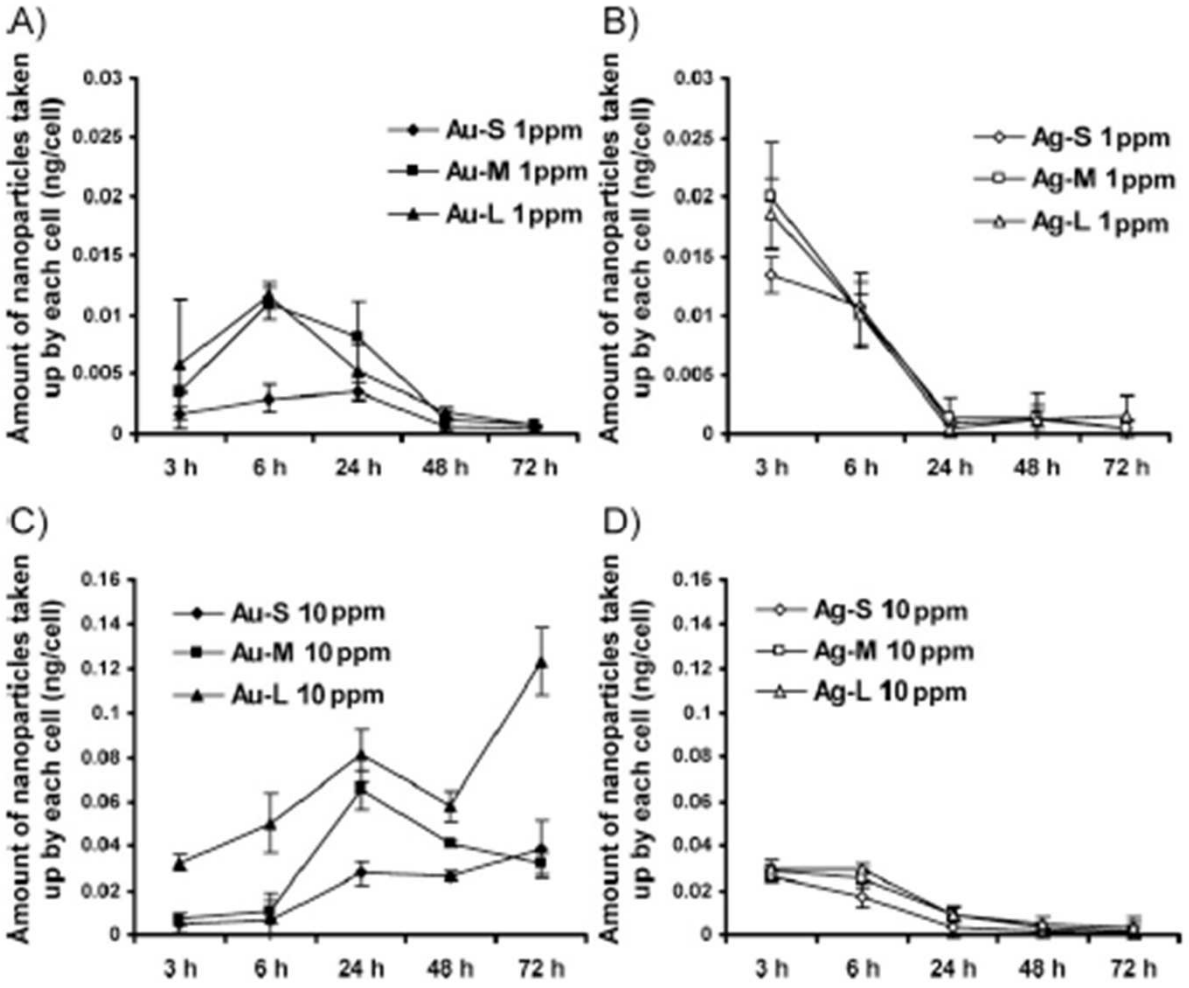


Figure 6. Cellular uptake of NPs as a function of incubation time for different sizes of AuNPs and AgNPs at 1 ppm (A and B) or 10 ppm (C and D).

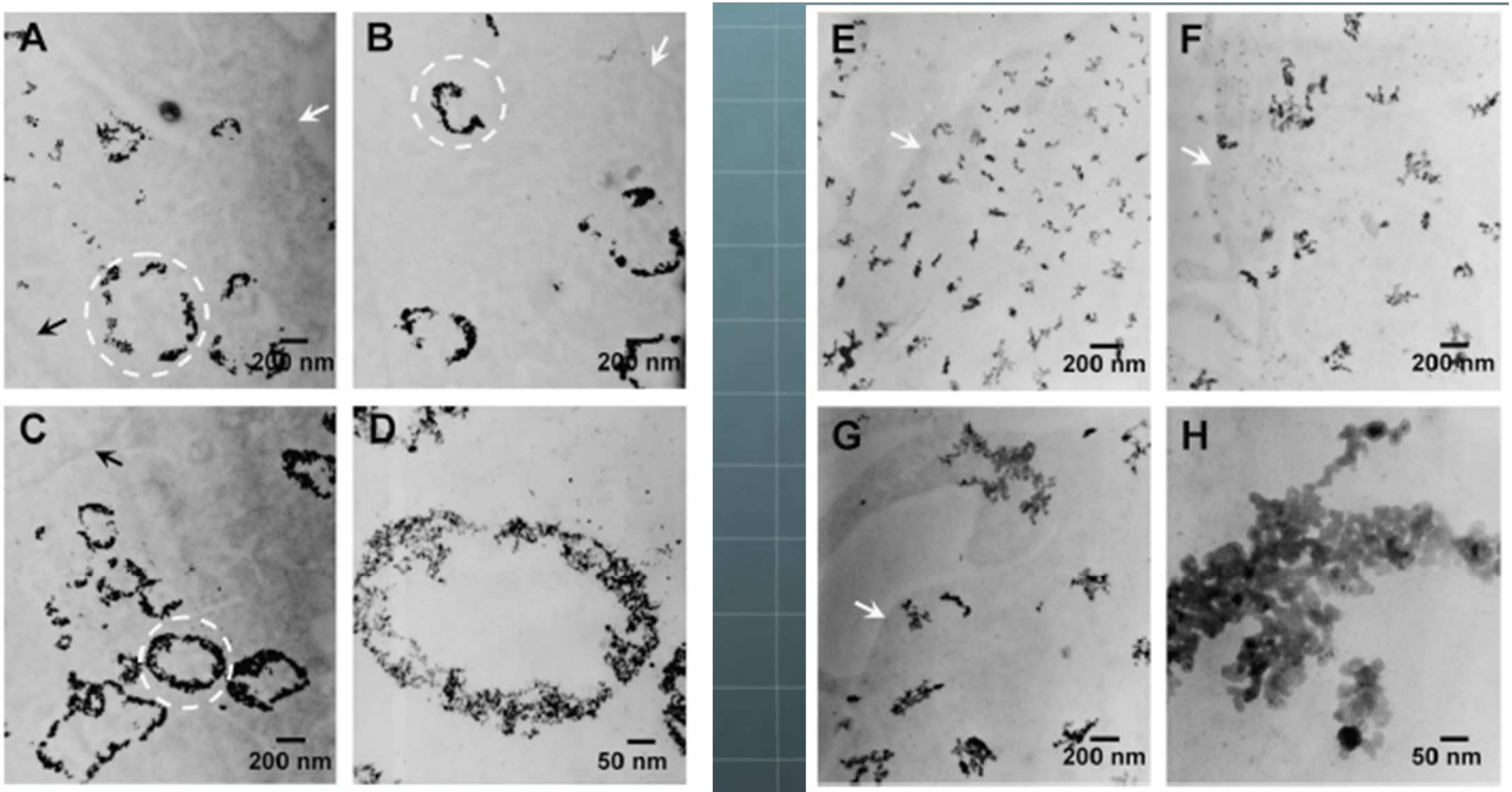


Figure 9. TEM images of macrophages incubated with AuNPs or AgNPs at 10 ppm for 24 h. A) Au-S, B) Au-L, C and D) Au-M, E) Ag-S, F) Ag-L, and G and H) Ag-M. Black arrows indicate the cell nuclei. White arrows indicate the cell membrane. Circles represent the receptor-mediated endocytotic vesicle.

Size

Table 1
Particle composition, size and surface area.

Particles	Description	Average size[a]	Size using TEM	Size in solution[b]	Surface area[c] [m^2/g]
Fe_2O_3 nano	Iron III oxide, nanopowder	29 nm	30–60 nm	1.6 μm	40[a]
Fe_2O_3 micro	Iron III oxide, powder, <5 μm, 99+%	<1 μm	0.15–1 μm		5.4
Fe_3O_4 nano	Iron II, III oxide, nanopowder, 98+%	20–30 nm	20–40 nm	<200 nm	42
Fe_3O_4 micro	Iron II, III oxide, powder, <5 μm, 98%	0.5 μm	0.1–0.5 μm		6.8
TiO_2 nano	Titanium IV oxide, nanopowder, 99.9%, mix of rutile and anatase	63 nm	20–100 nm	300 nm	24[a]
TiO_2 micro	Titanium IV oxide, powder <5 μm, 99,9%, rutile, contains small amount of anatase	1 μm	0.3–1 μm		2.5
CuO nano	Copper II oxide, nanopowder	42 nm	20-40 nm	200 nm	23[a]
CuO micro	Copper II oxide, powder, <5 μm, 98%	3 μm	0.5-10 μm		1.5[d]

[a] According to the manufacturer Sigma–Aldrich.
[b] Using dynamic light scattering (DLS).
[c] Using BET (Brunauer, Emmett, Teller) analysis.
[d] Analyzed by Klara Midander and Inger Odnevall Wallinder, The Royal Institute of Technology, Stockholm, Sweden.

Karisson et al 2009

Size

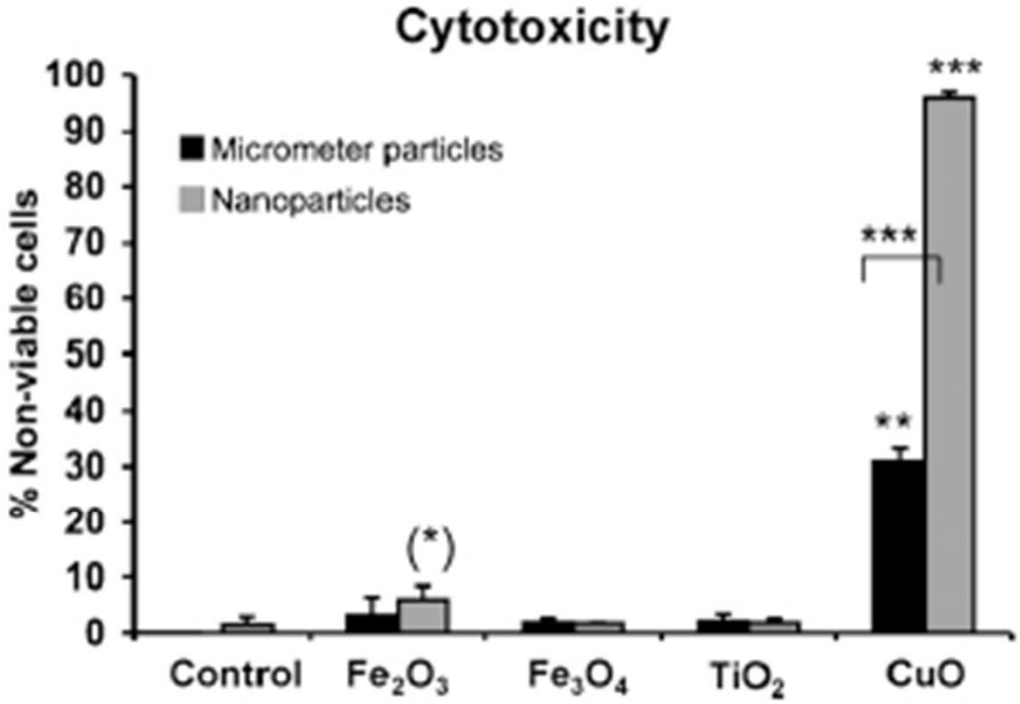
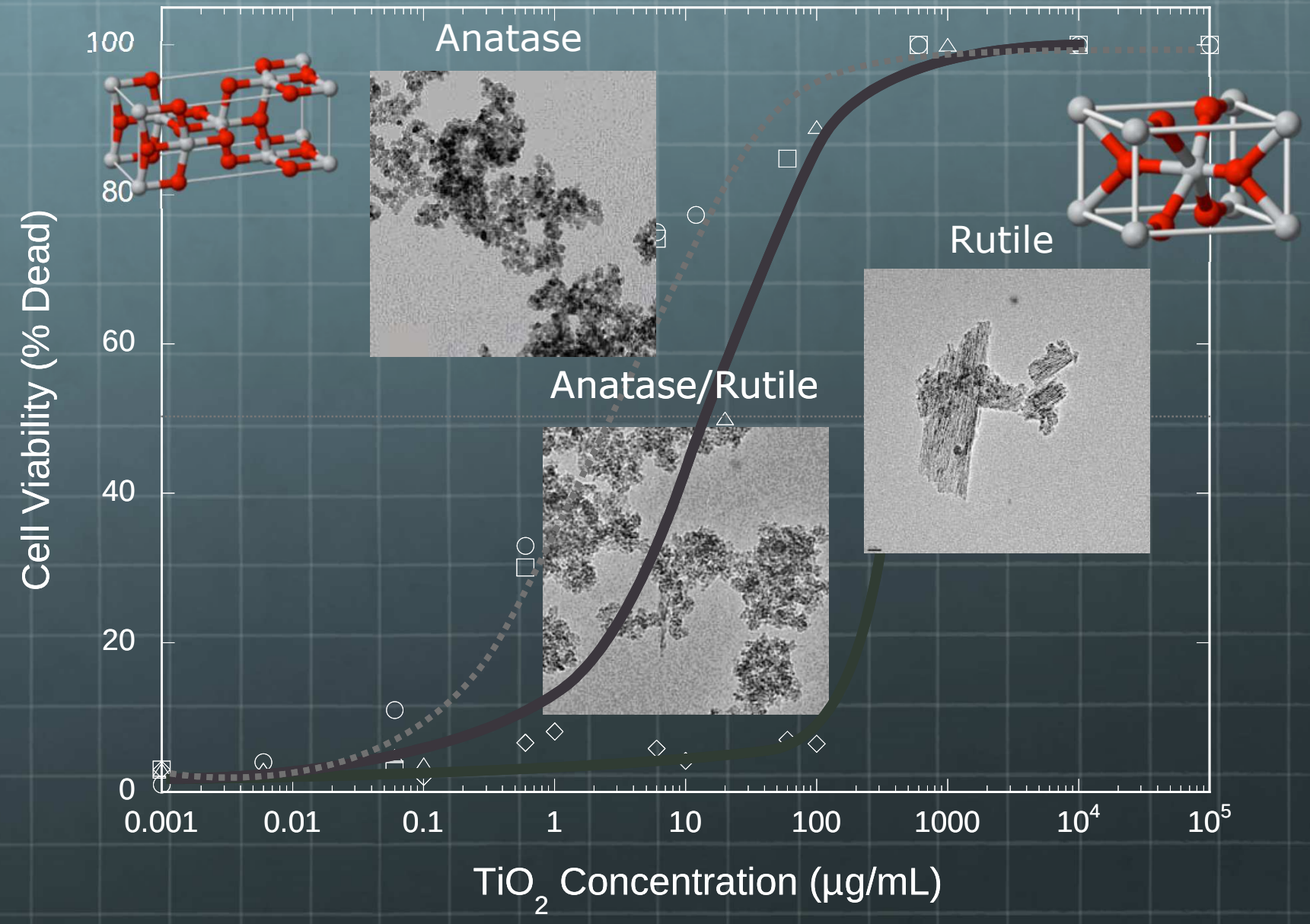


Fig. 2. Cell viability of A549 cells after 18 h exposure to 40 μg/cm² nano- or micrometer particles of different composition. Cytotoxicity was measured as percent non-viable cells by trypan blue staining. Each bar represents the average value of three independent experiments ± SD. Stars (*, ** and ***) indicate significantly higher levels compared to controls, and correspond to $p < 0.05$, 0.01 and 0.001. Nanoparticles of CuO showed a significantly higher value ($p < 0.001$) of cytotoxicity than micrometer particles.

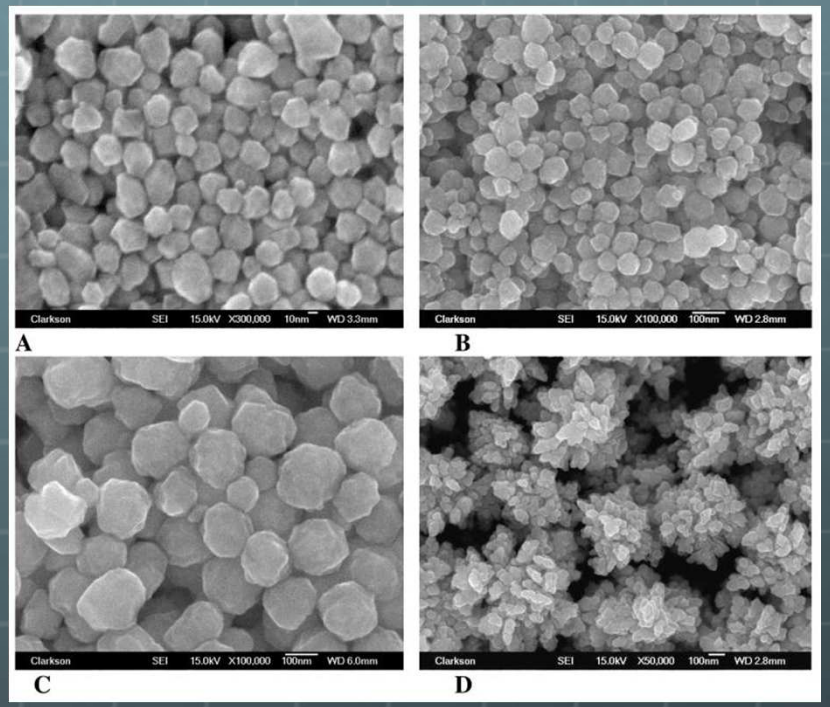
Structure (shape)-related hazard: Crystallinity

In vitro studies - Human Dermal Fibroblasts



Shape

Ispas et al. 2009

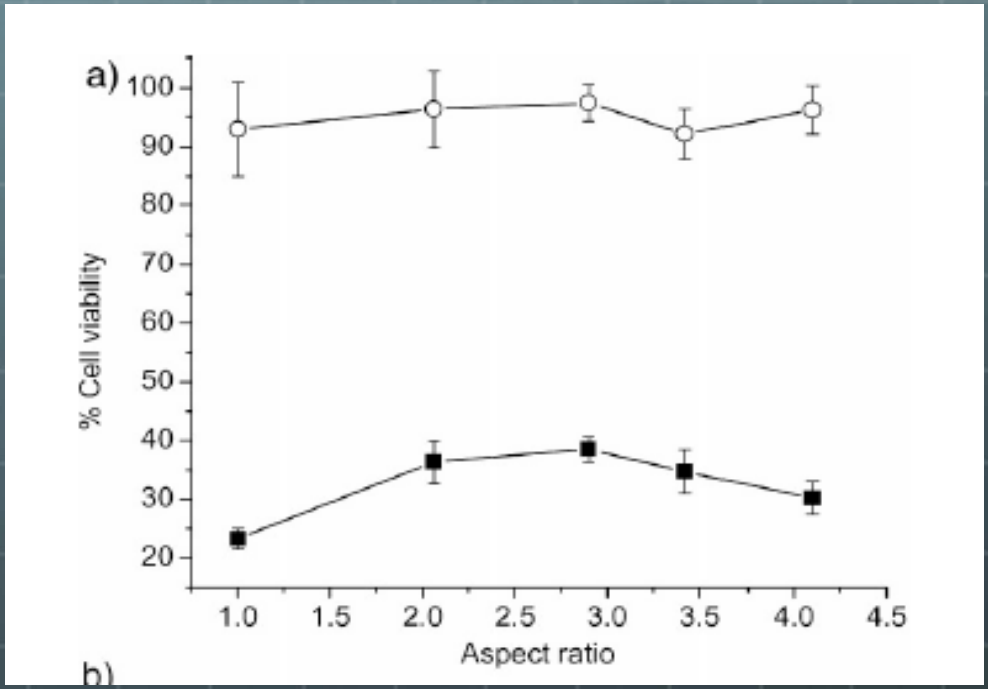


FE-SEM images of Ni NPs with an average particle diameter of 30 nm (A), 60 nm (B), 100 nm (C), and dendritic structures with aggregated 60 nm entities (D).

Nickel	LD10	LD50
30 nm Spheres	187 (143–229)	328 (299–357)
60 nm Spheres	189 (111–264)	361 (315–404)
100 nm Spheres	172 (140–188)	221 (212–231)
Dendritic particles of aggregated 60 nm spheres	21 (9–43)	115 (90–168)
Soluble nickel	63 (40–96)	221 (181–271)

Toxicity of Ni nanoparticles and soluble Ni on zebrafish embryos in mg/L. LD10= 10% lethal concentration and LD50= median lethal concentration. Values in parentheses are 95% confidence intervals.

Shape



Cell viability as a function of aspect ration for CTAB coated nanorods (dark squares) and PAA coated nanorods (white circles)

- Alkilany et al. 2009 - Change in aspect ratio for gold NPs does not significantly affect toxicity