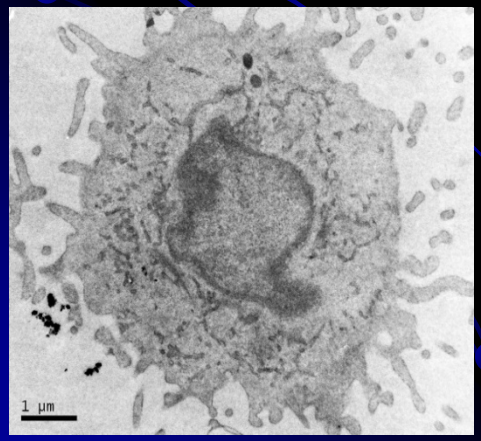
SRC/SEMATECH Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Characterization and Systematic Evaluation of the Toxicity of Metal-based Nanoparticles



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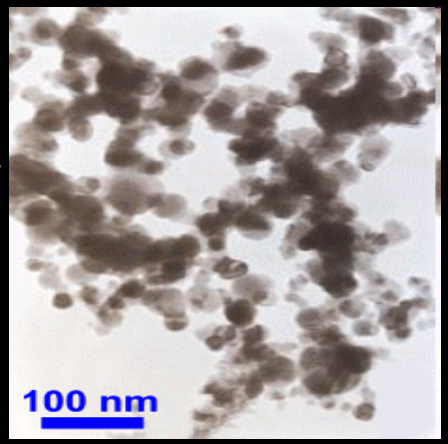
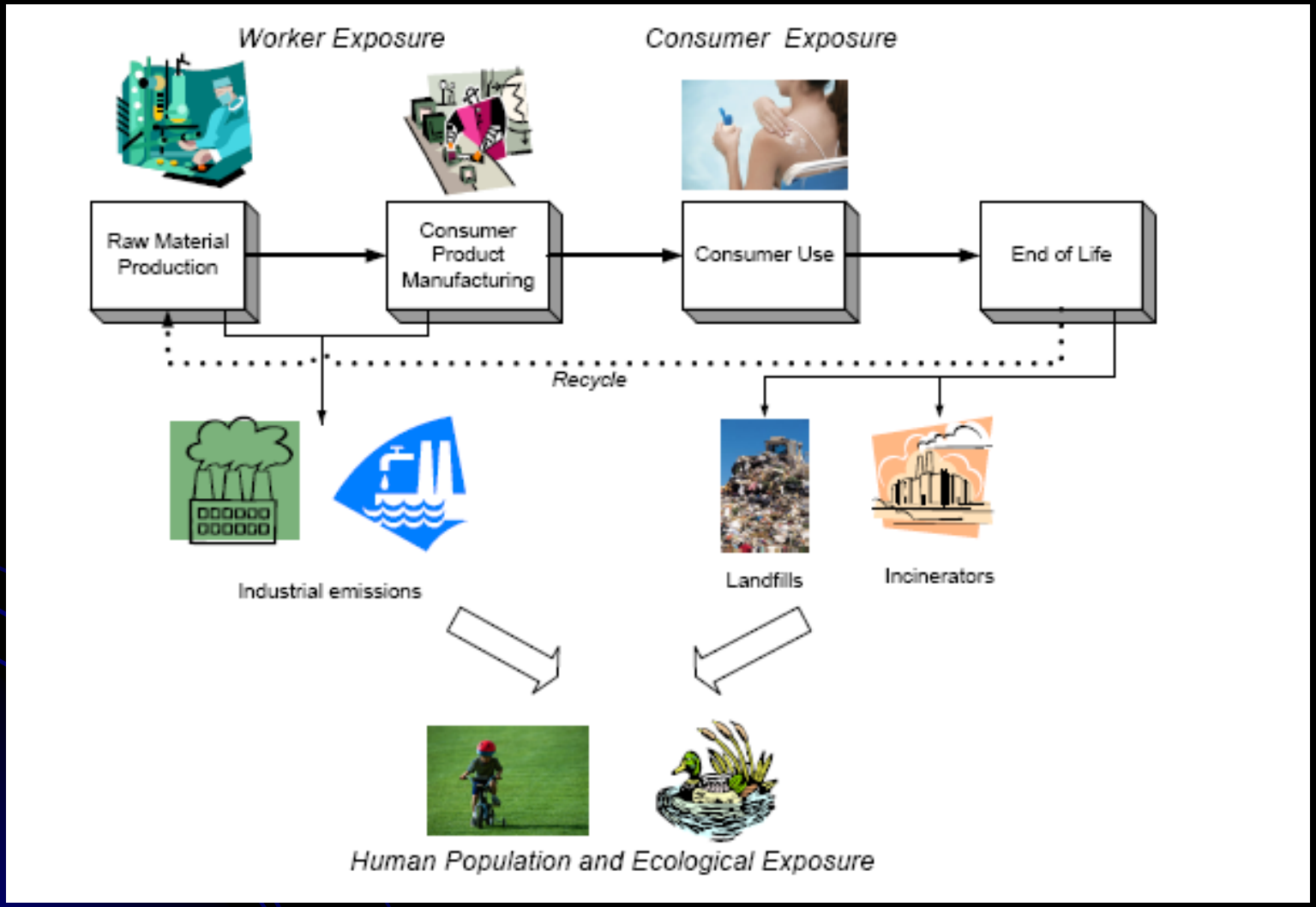


Nanomaterial Exposure Cycle



US EPA White Paper on Nanotechnology, 2007

PM_{10} Air Pollution Linked to Human Disease

Oxidative Stress ➔ Inflammation ➔ Disease (i.e., cancer, CVD, lung)

Diesel exhaust particulate

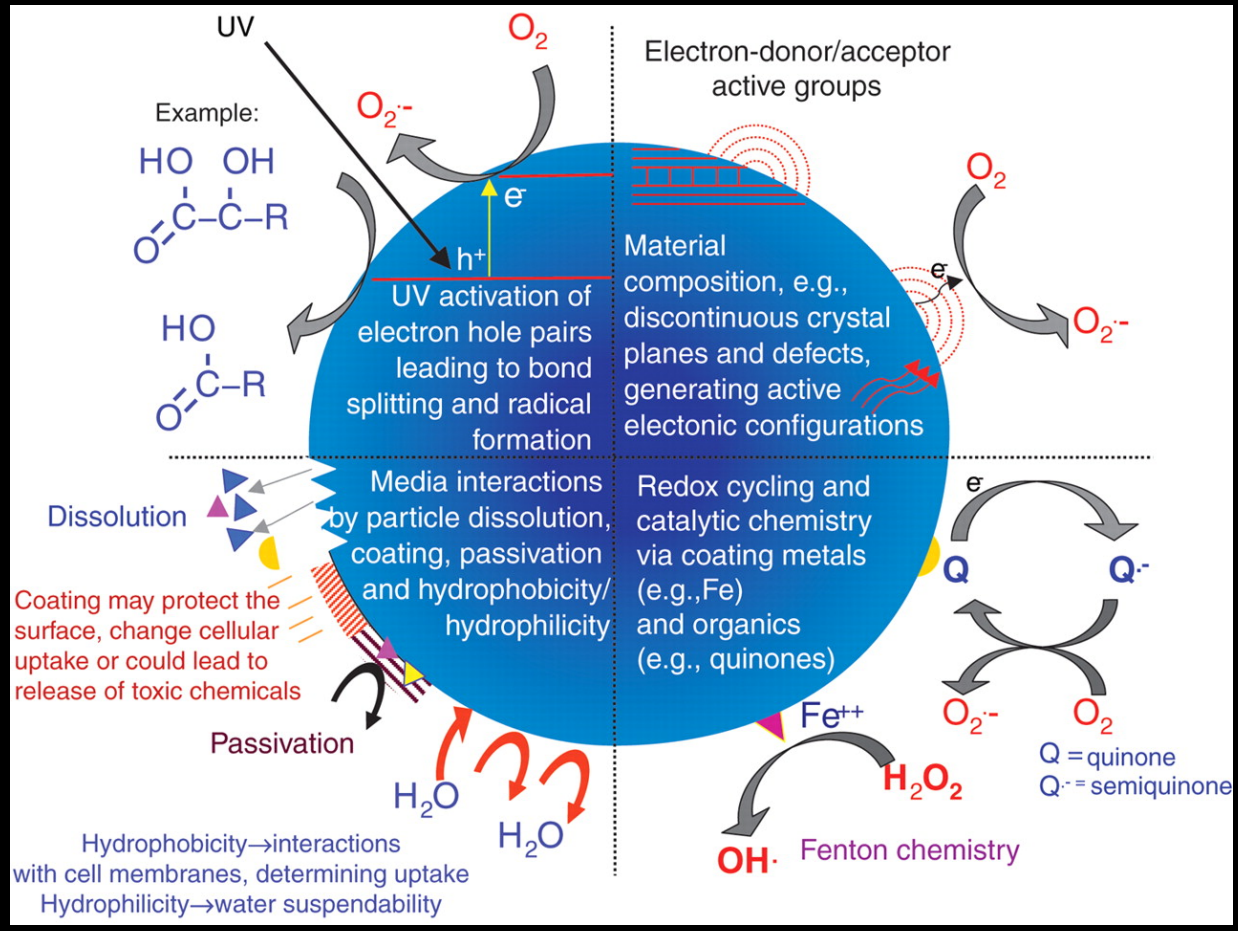
Coal fly ash

Nanoparticulate carbon black

Donaldson et al., Combustion-Derived Nanoparticles: A Review of Their Toxicology Following Inhalation Exposure. ***Part. Fibre Toxicol. 2005***

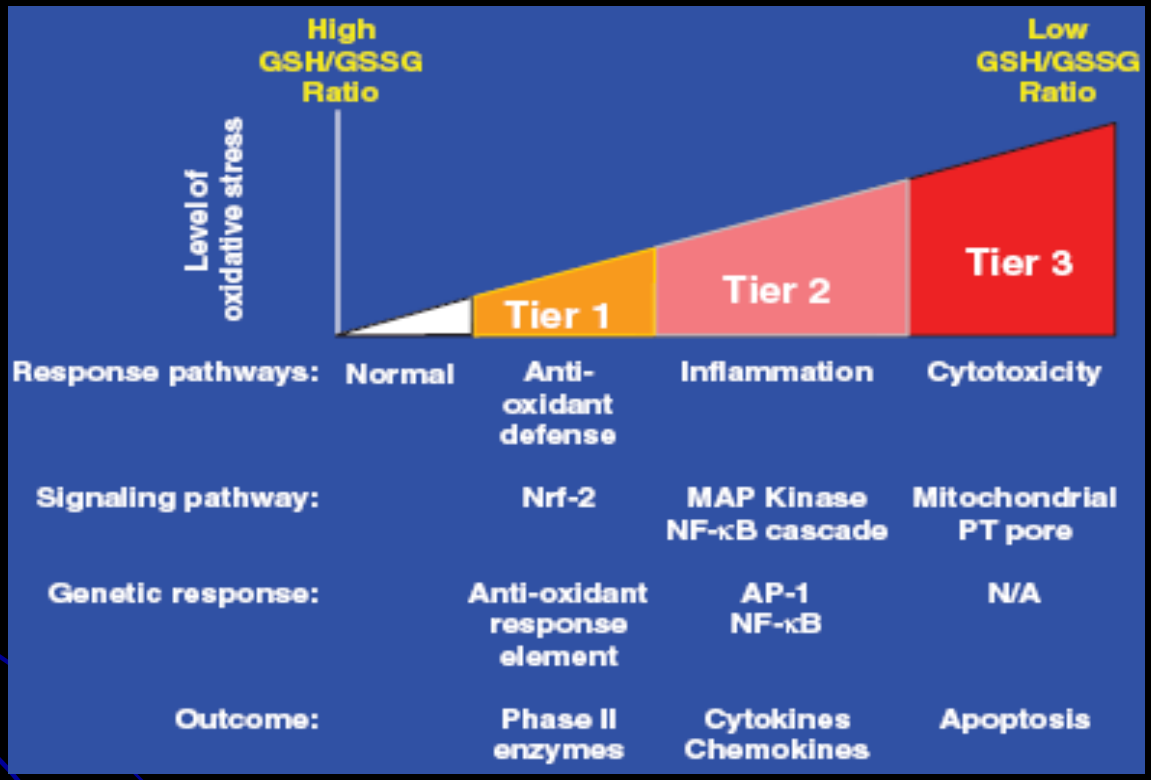
Challenges - Nanoparticles

- **Composition**
- **Size**
- **Shape**
- **Biopersistence**
- **Surface chemistry**
- **Release of chemicals**



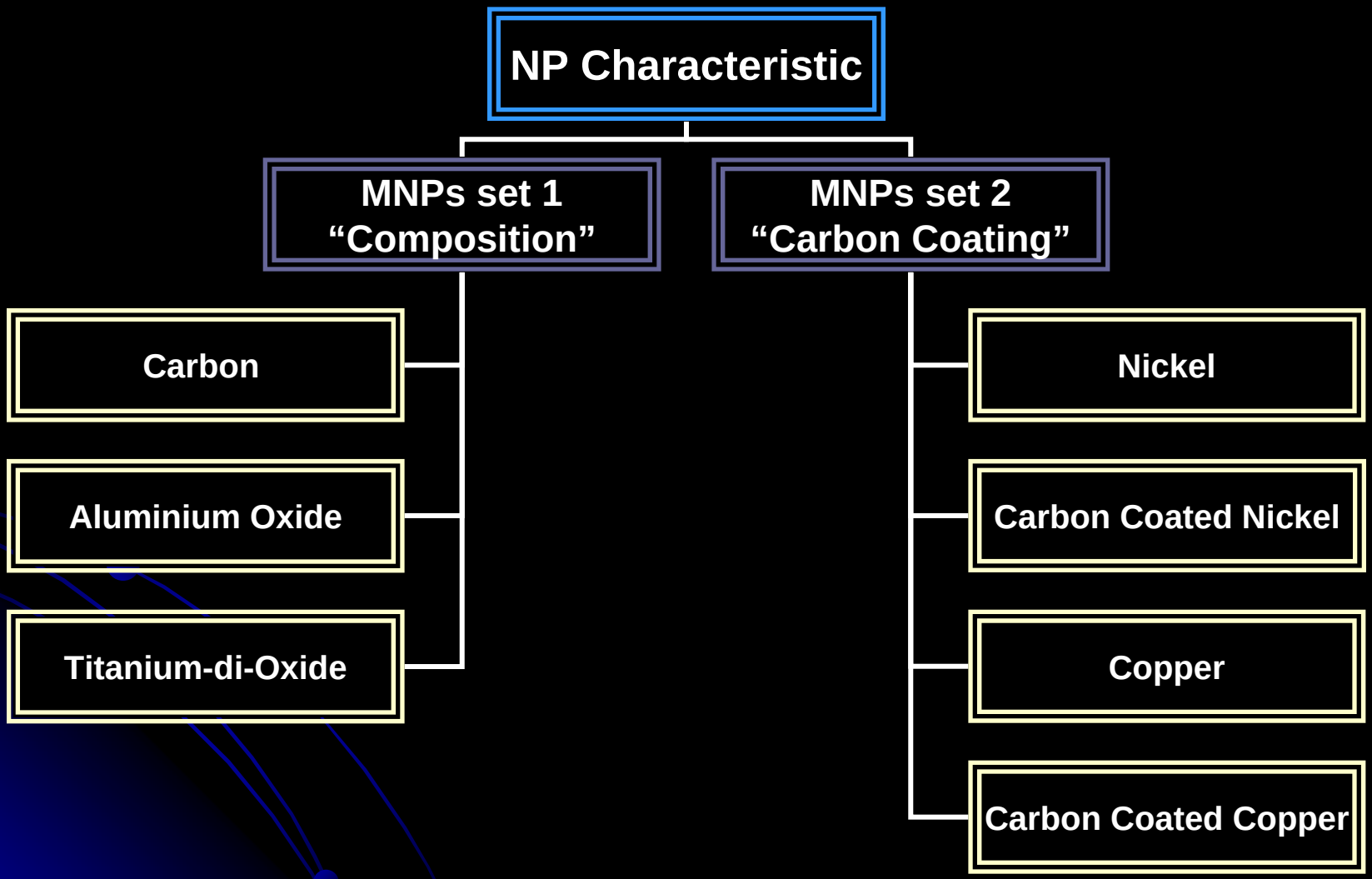
Nel et al., *Science,* 2006

Oxidative Stress Paradigm



Nel et al., *Science,* 2006

Manufactured Nanoparticles (MNPs)



Hypothesis

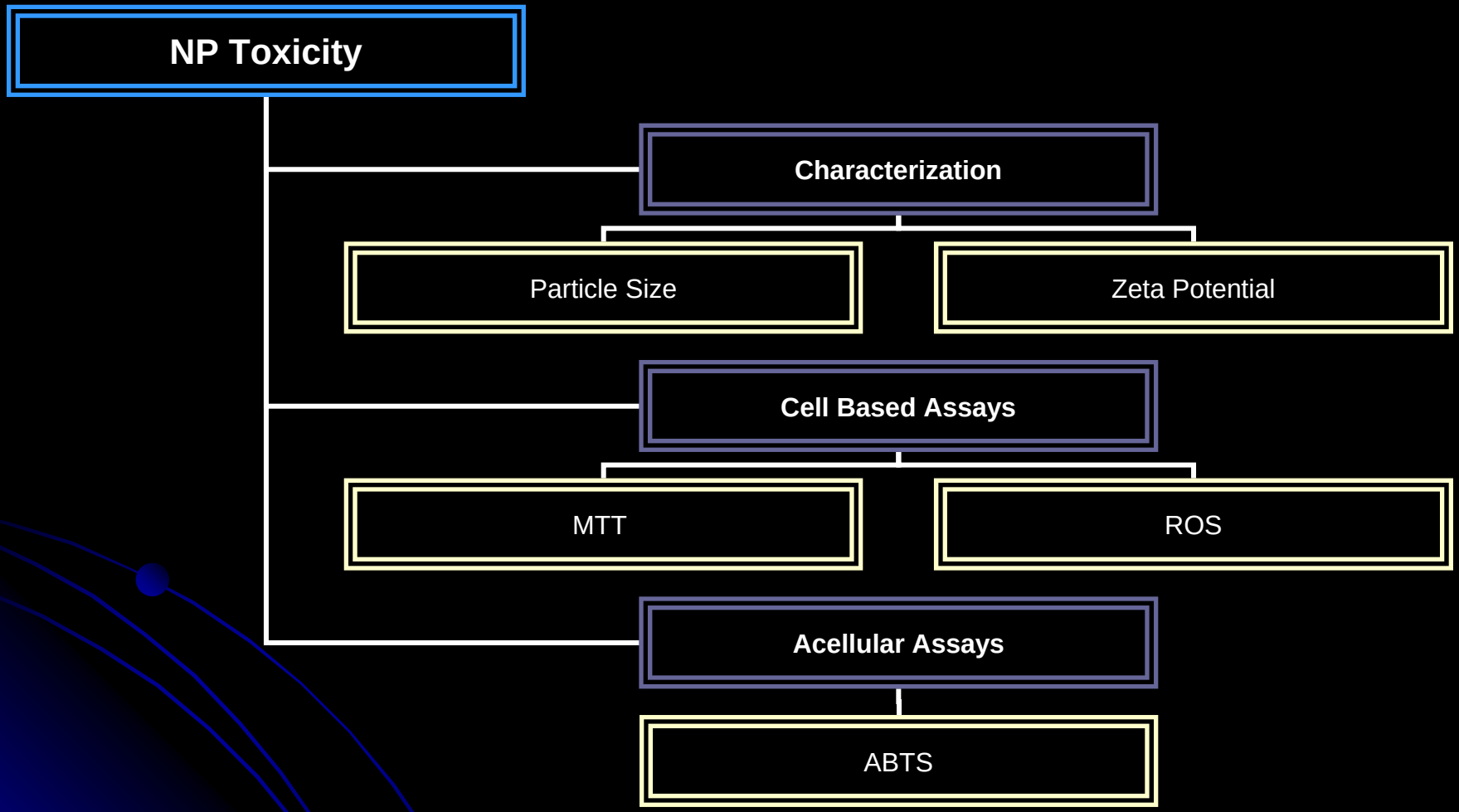
- Manufactured nanoparticles Set 1 (MNPs Set 1) with different compositions but similar size range will exhibit different toxicity profiles when screened for their toxic effects via *in-vitro* acellular and cell based assays.

- Differences in toxicity observed with MNPs Set 1 will depend on the inherent composition and/or impurities associated with the NPs.

- Amongst matched set of carbon coated and bare metal based nanoparticles (MNPs Set 2), carbon coated metal nanoparticles will be more toxic in cell based assays as compared to bare metal NPs.

- Relatively higher toxic potential of carbon coated metal nanoparticles will be attributable to their hydrophobic nature and their capability to carry higher payload of metal NPs inside cells.

Scheme - Toxicity Testing (MNPs Set 1)



Cell Line Model:
A549 alveolar epithelial cells

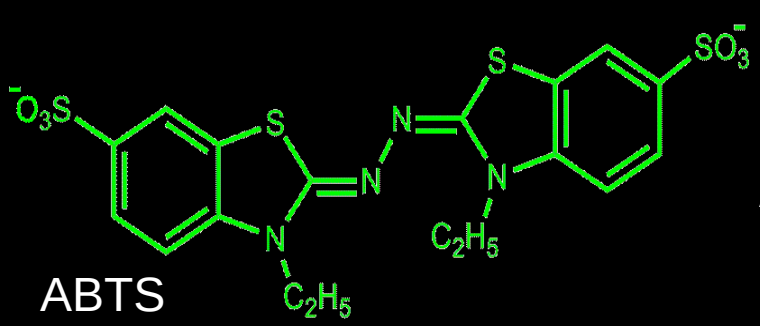
MNPs Set 1 - Different Compositions

NP Type	Manufacturer	Particle Size* Range (nm)	Particle Size in DI water (nm)	Zeta Potential (mV)
Carbon	American Elements	55-100	400-500	-21.1 ± 4.6
Aluminum oxide	Alfa-Aesar	40-50	330-430	-17.7 ± 7.4
Titanium-di-oxide	NanoAmor	30-40	400-500	-25.3 ± 5.2

* Provided by Manufacturer

Sample preparation:
1 mg/ml suspensions in DI water, bath sonicated for 6 X 30 sec

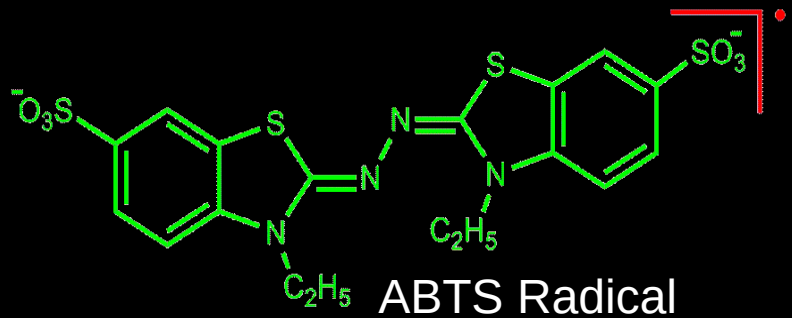
Acellular ABTS Assay



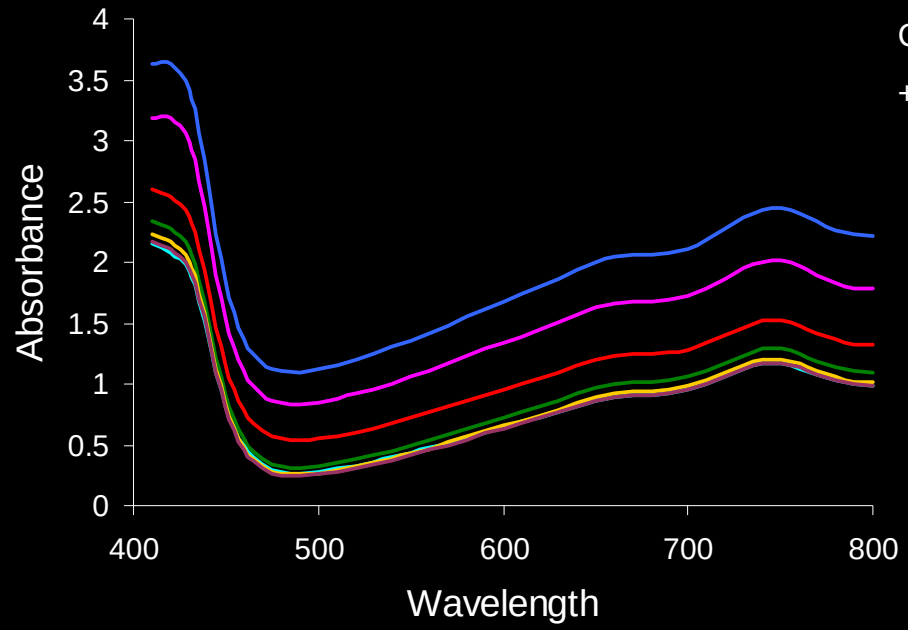
NPs

$K_2S_2O_8$

Antioxidant



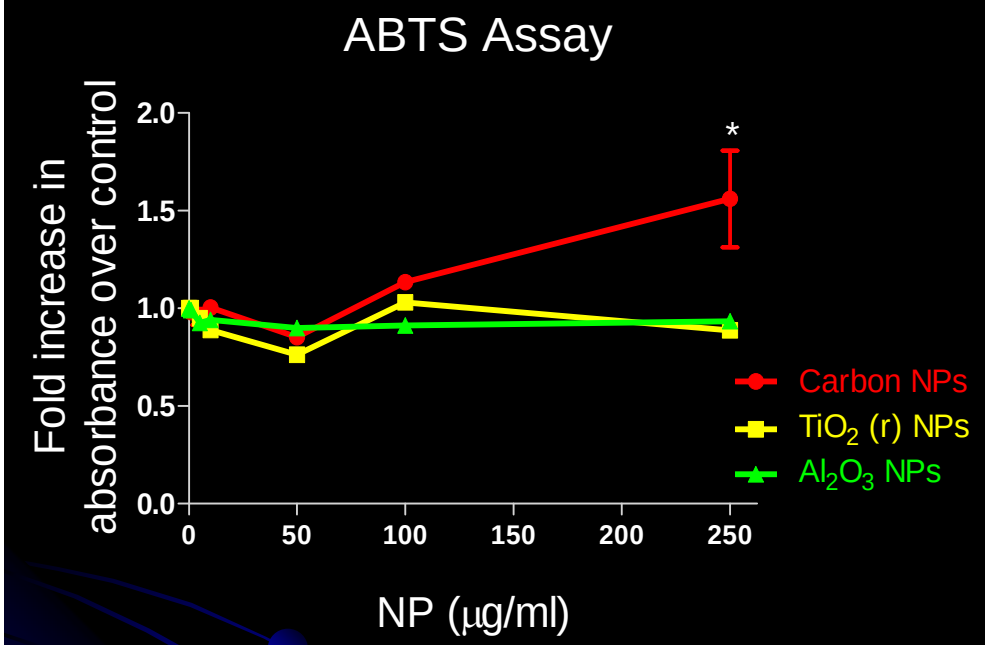
ABTS Radical
(Abs. at 734 nm)



Wavelength vs. Absorbance for Carbon NP induced ABTS radical (not corrected for blank NPs)

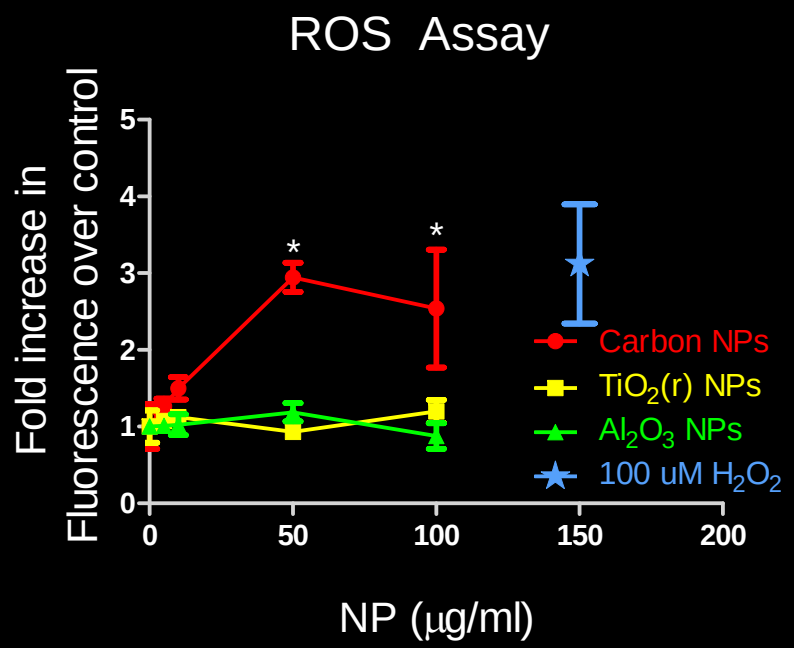
ABTS = 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)

ABTS and ROS Assay



- Assay in 96 well plate, ABTS 60 mM
- Incubation with NPs for 24 hr
- Data corrected for absorbance from blank NPs

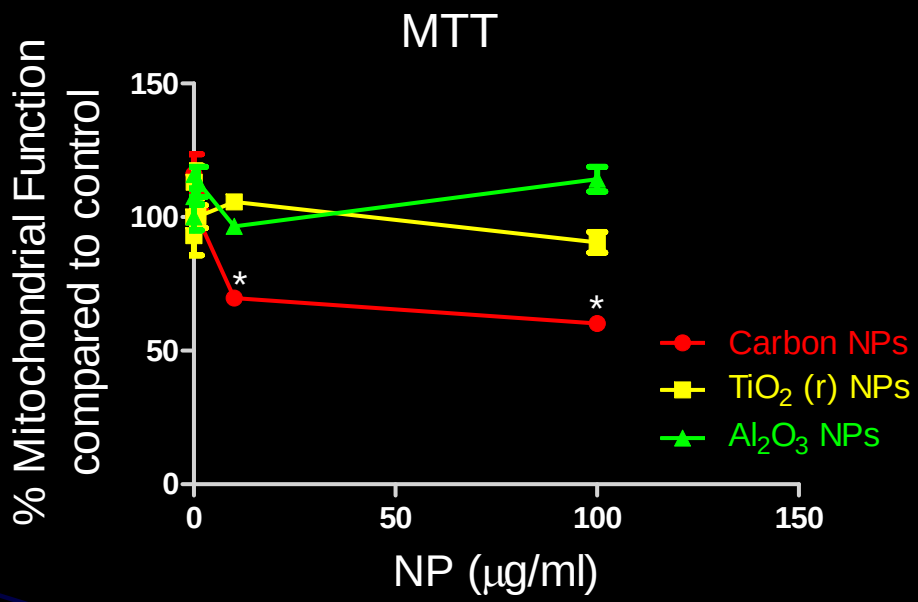
(* $p < 0.05$ as compared to control)



- A549 cells (25,000 per well)
- Incubation with NPs for 4 hr
- Data corrected for fluorescence from blank NPs
- H_2O_2 as positive control

(* $p < 0.05$ as compared to control)

Mitochondrial Function



Analysis	%
Si	O.1
Al	0.005
Na	0.0008
Cr	0.06
Ni	0.05
Ca	0.01
Fe	0.08
F	0.03

- A549 cells (25,000 per well)
- Incubation with NPs for 24 hr
- Data corrected for absorbance from blank NPs

(* $p < 0.05$ as compared to control)

Impurities in Carbon NPs from certificate of analysis provided by the manufacturer.

Explanation: Carbon nanoparticles act as a vehicle to carry iron/nickel in cells. This is a very plausible scenario as hydrophobicity provided by carbon NPs facilitate iron entry in cells.

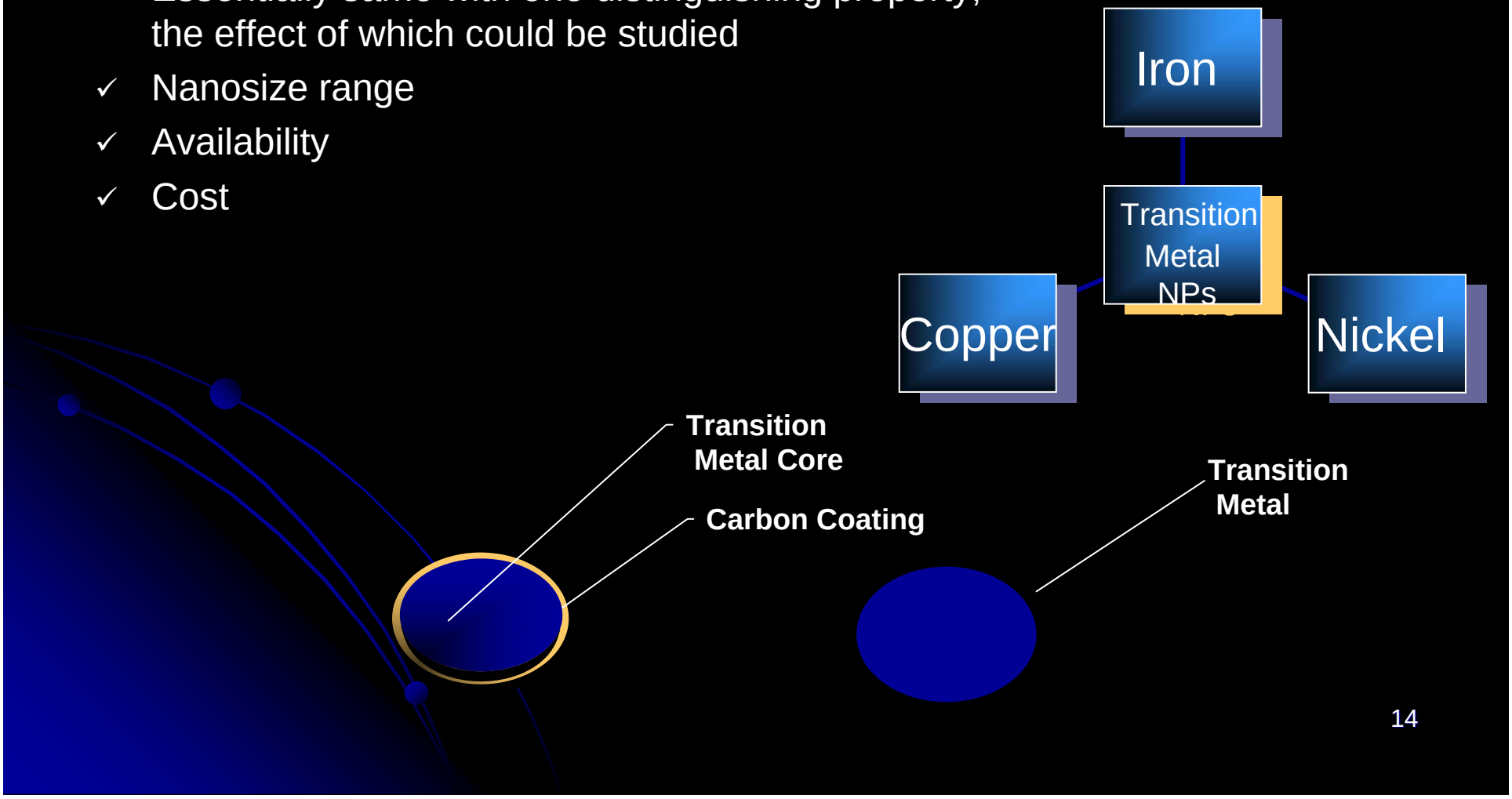
Conclusions - MNPs Set 1

- Particle size measurement by dynamic light scattering shows that NP sizes are different from those provided by the manufacturer.
- ABTS assay was successfully developed as an *in-vitro* acellular assay to assess the free radical forming potential of NPs. The assay is simple, adaptable to 96 well plate and cost effective.
- Carbon NPs appear to be more toxic as compared to other NPs as shown by in-vitro cytotoxicity and ROS data.
- Results from ABTS assay correlates well with ROS and MTT cytotoxicity data.

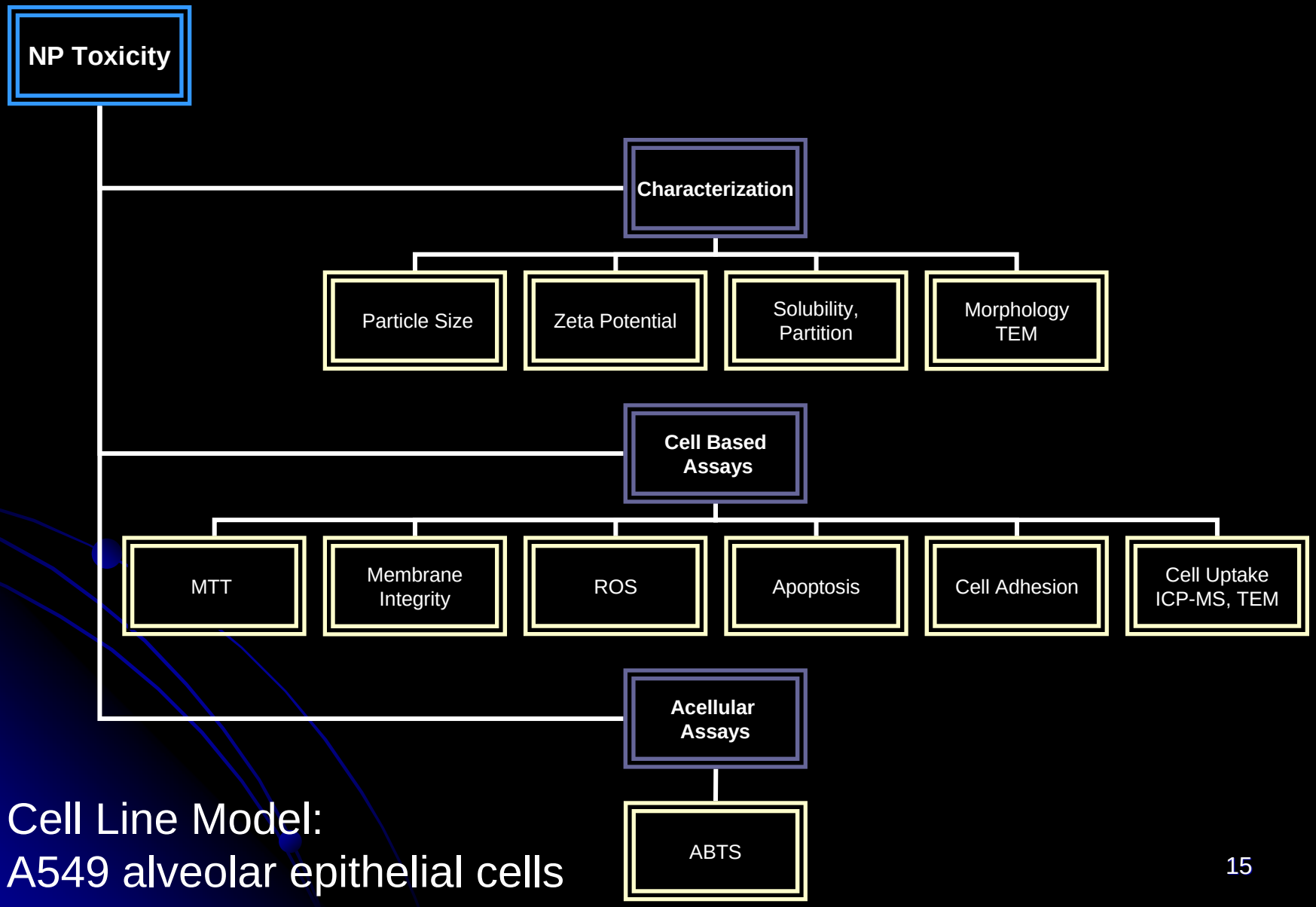
Strategy - MNPs Set 2

- Desirable features of MNPs for systematic toxicity testing.

- ✓ Essentially same with one distinguishing property, the effect of which could be studied
- ✓ Nanosize range
- ✓ Availability
- ✓ Cost



Scheme - Toxicity Testing



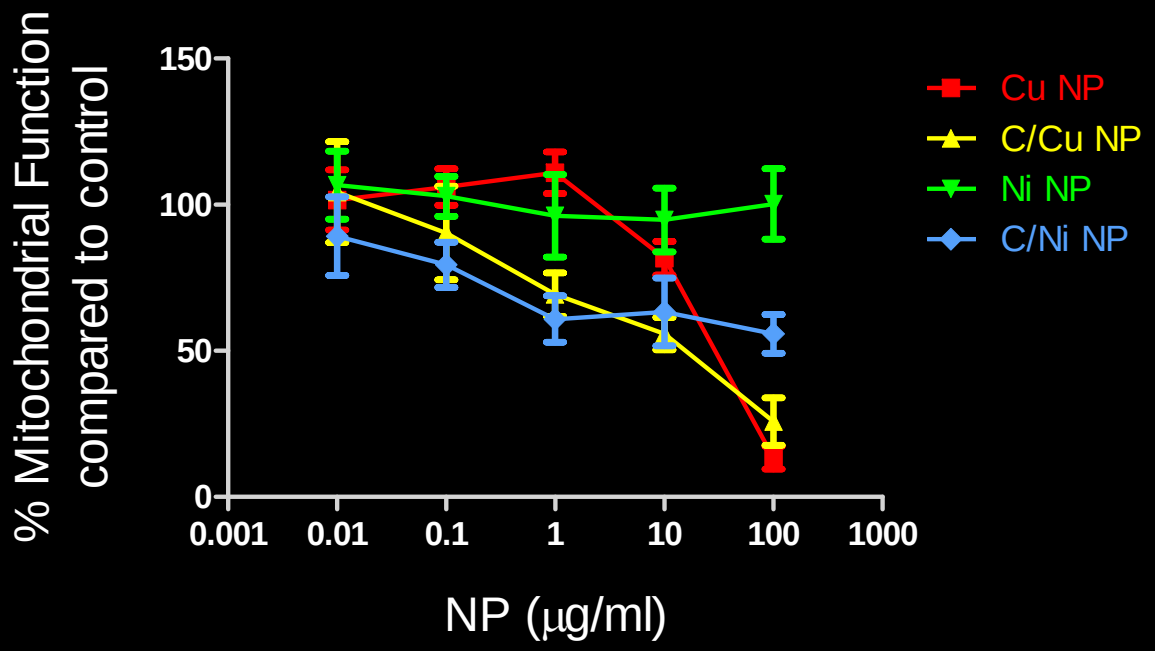
Cell Line Model:
A549 alveolar epithelial cells

MNPs Set 2 – Matched Sets of Carbon Coated and Bare Metal MNPs

NP Type	Manufacturer	Particle Size* Range (nm)	Particle Size in DI water (nm)	Zeta Potential (mV)
Nickel	NanoAmor	20	834.63 ± 495.13	2.76 ± 0.74
Carbon coated Nickel	NanoAmor	20	466.63 ± 179.63	-16.4 ± 1.83
Copper	NanoAmor	25	662.17 ± 139.3	-9.0 ± 2.4
Carbon coated Copper	NanoAmor	25	412.13 ± 210.88	-6.21 ± 0.73

* Provided by Manufacturer

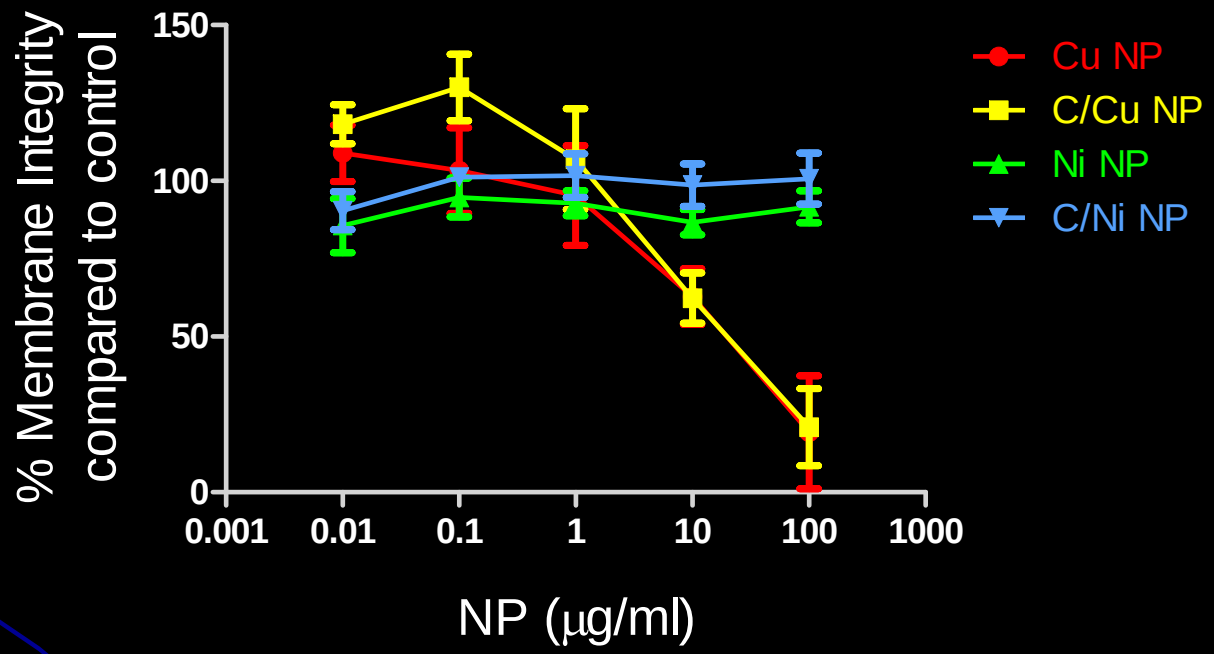
MTT Assay - MNPs Set 2



- A549 cells (25,000 per well), incubation with NPs for 24 hr
- Data corrected for absorbance from blank NPs
- Ni NPs significantly differ from C/Ni NPs at all doses
- Cu NPs significantly differ from C/Cu NPs at 0.1, 1 and 10 µg/ml

($p < 0.05$ as compared to control)

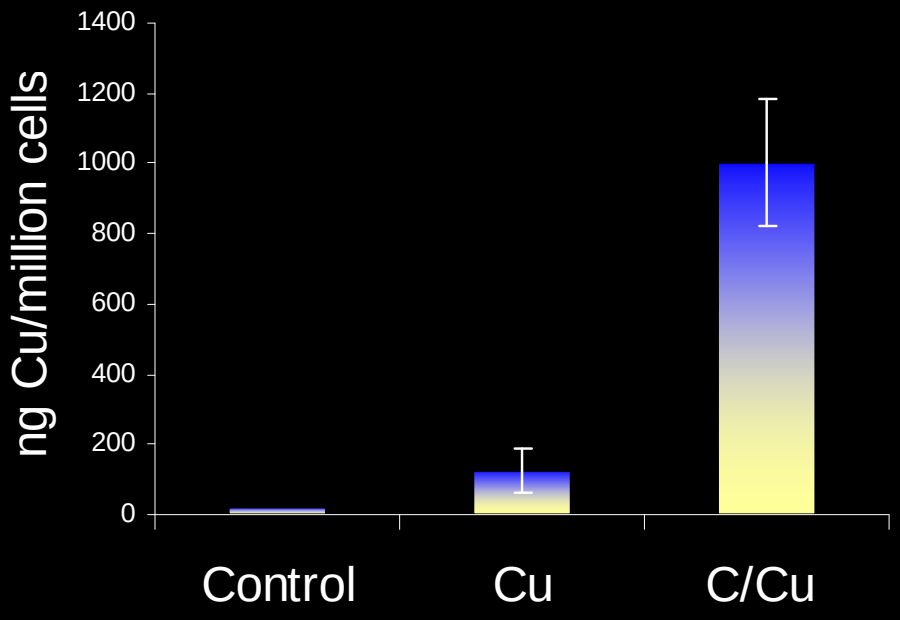
Neutral Red Membrane Integrity Assay - MNPs Set 2



- A549 cells (25,000 per well), incubation with NPs for 24 hr
- Data corrected for absorbance from blank NPs
- Ni NPs and C/Ni NPs do not alter membrane integrity
- Cu NPs and C/Cu NPs are equally toxic

Cell Uptake Studies By ICP MS: Cu and C/Cu NPs

- MNPs: Cu and C/Cu NPs
- Dose: 10 µg/ml
- Time point: 8 hr
- Corrected for
  - Cu and C/Cu blanks
  - Basal level of Cu
  - Matrix effect

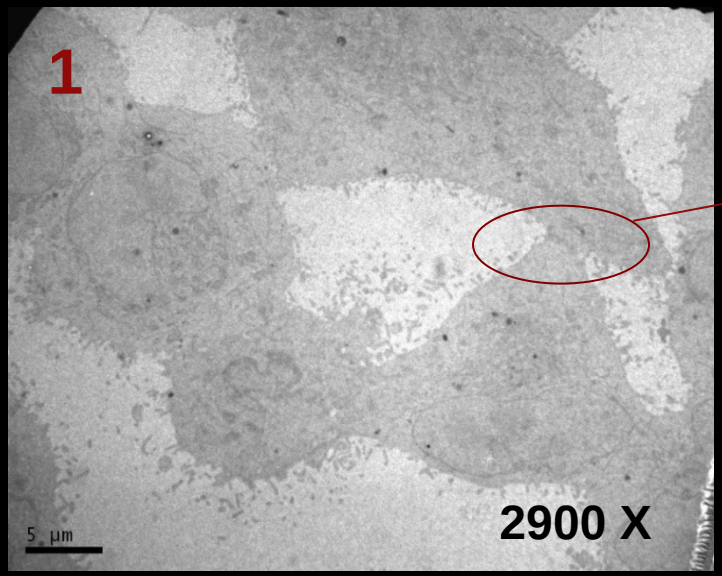


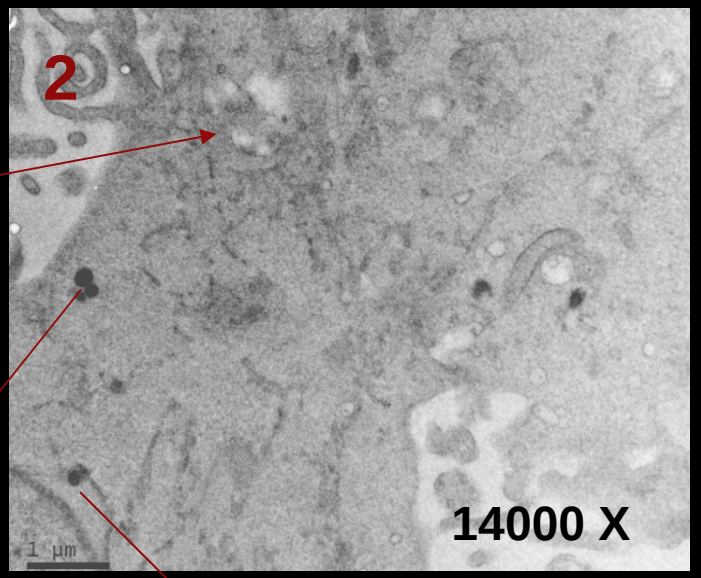
Cell Uptake Studies By TEM

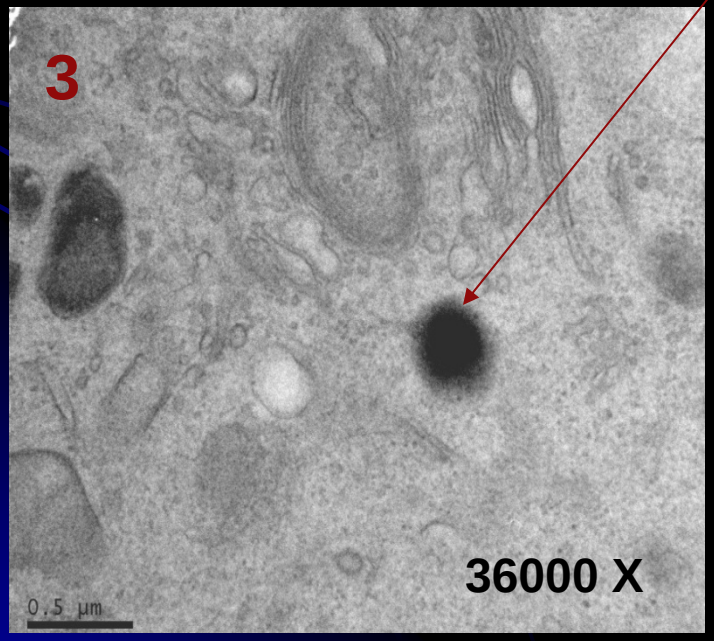
- MNPs: Cu, C/Cu, Ni and C/Ni NPs
- Dose: 10 µg/ml
- Time point: 8 hr
- Control: untreated A549 cells

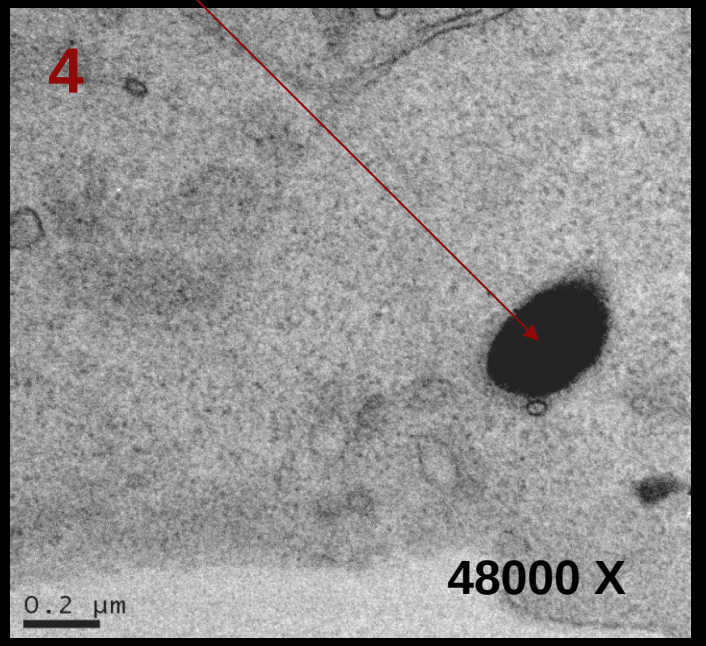
TEM: JEOL 100 CX II

Control: A549 Cells

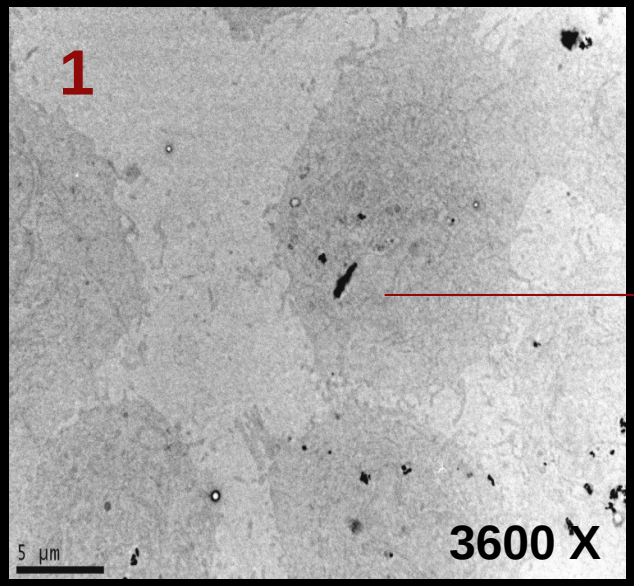


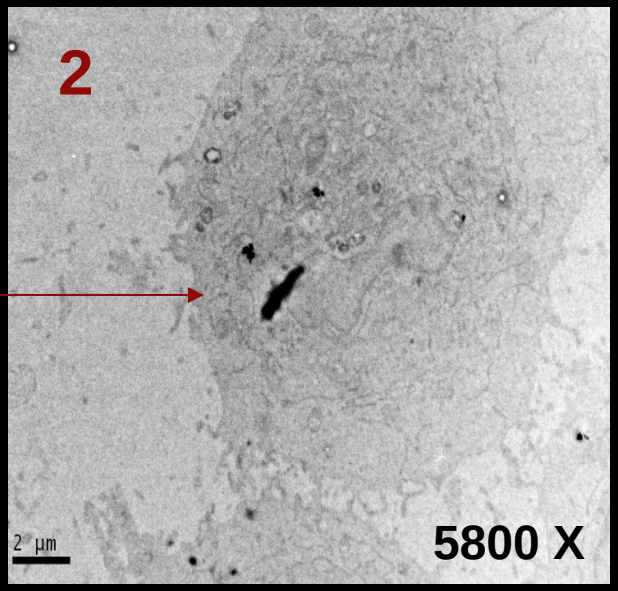


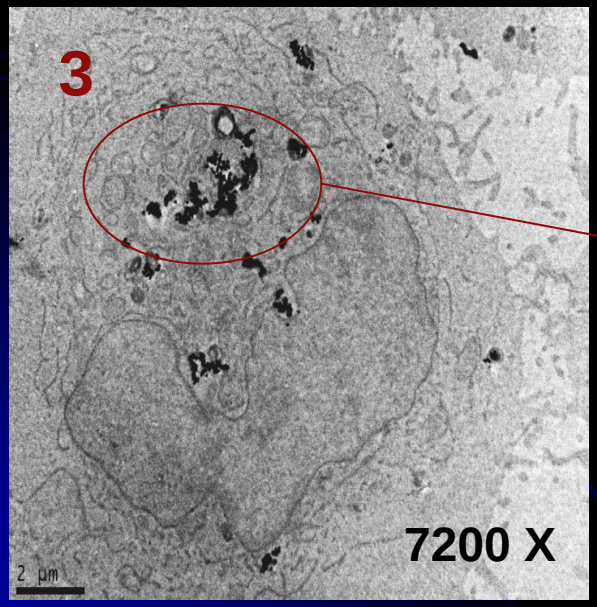


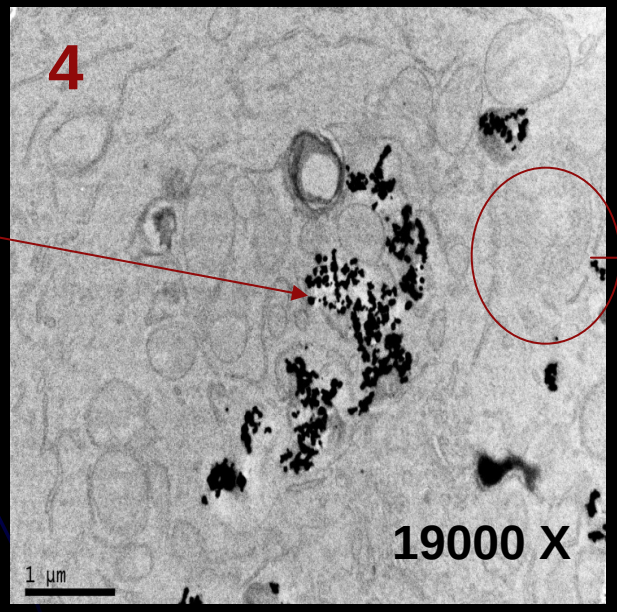


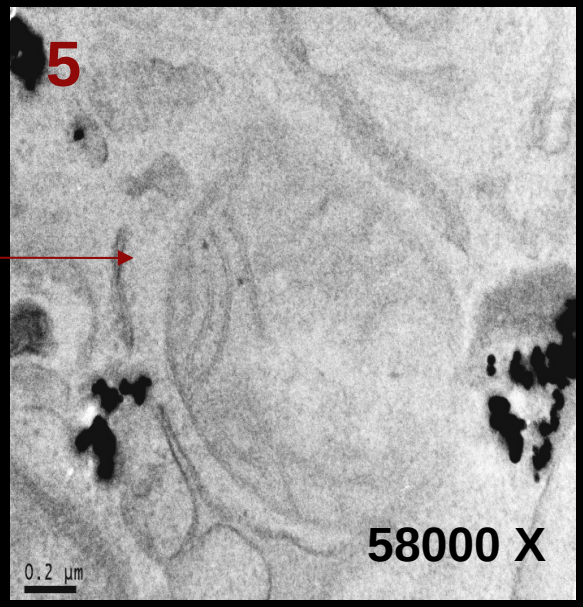
Cu NP Treated A549 Cells



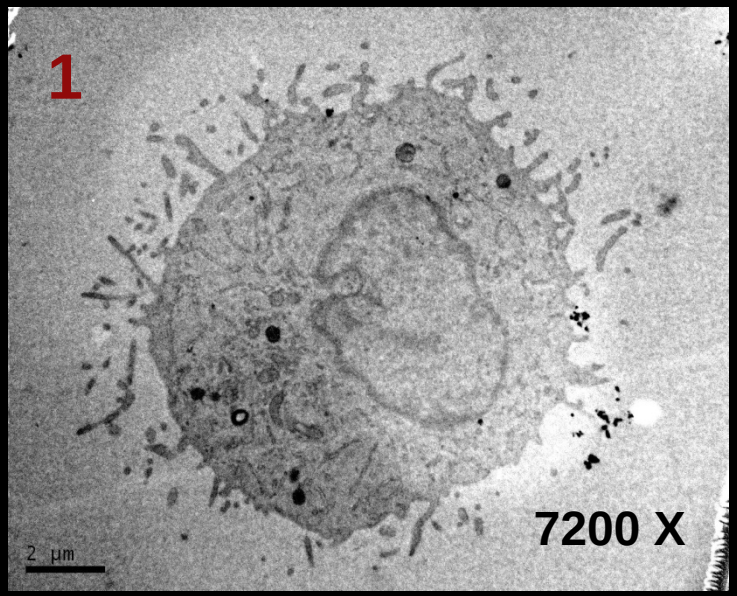


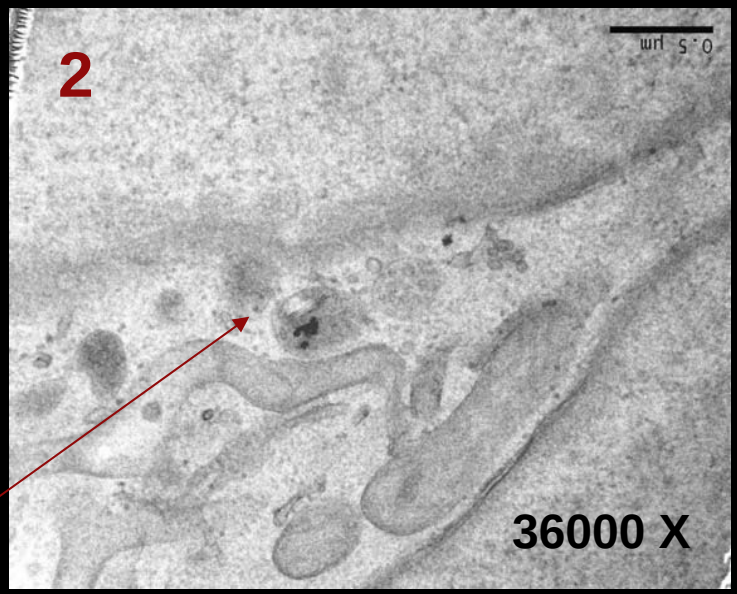


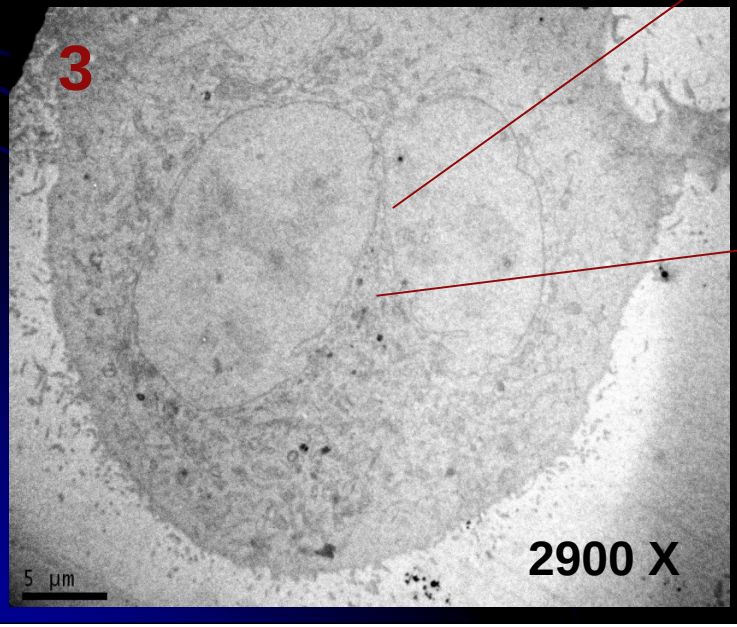


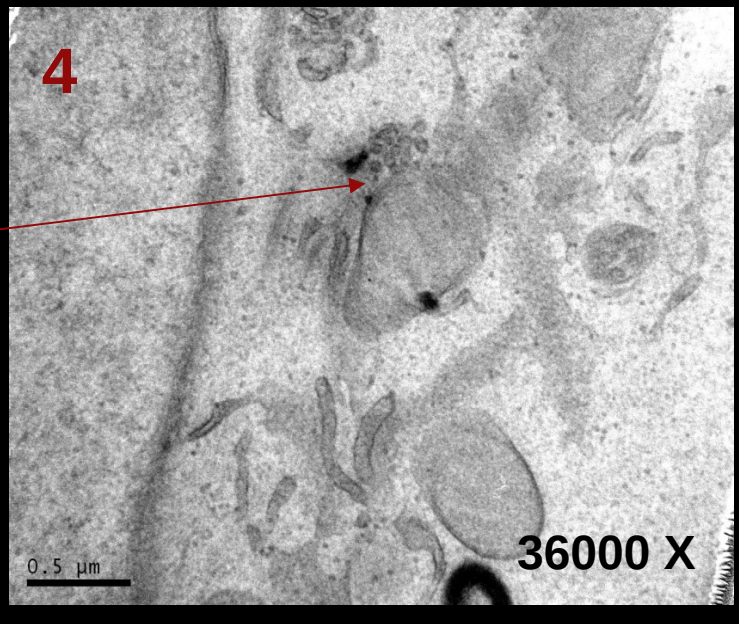


C/Cu Treated A549 Cells

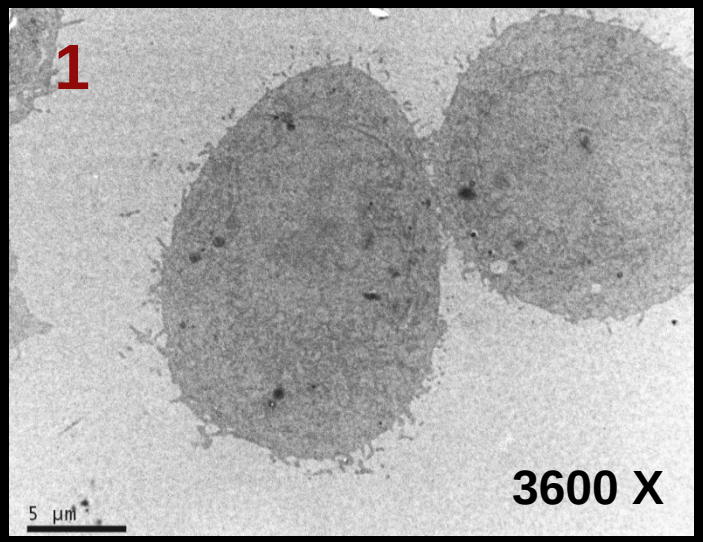


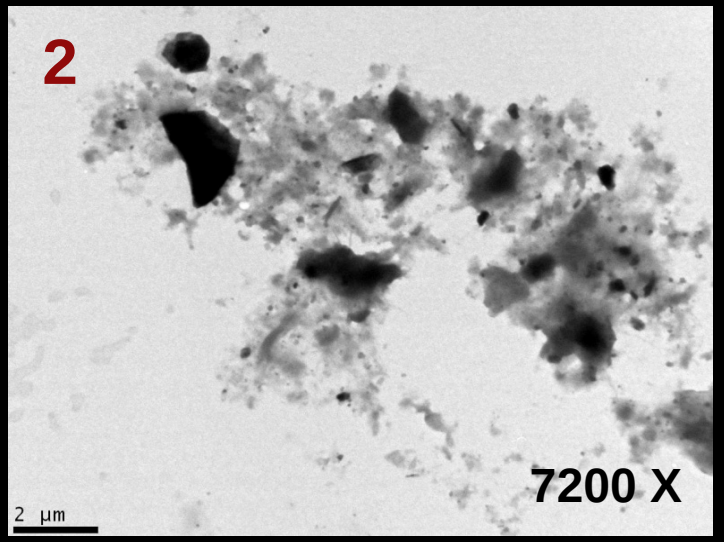


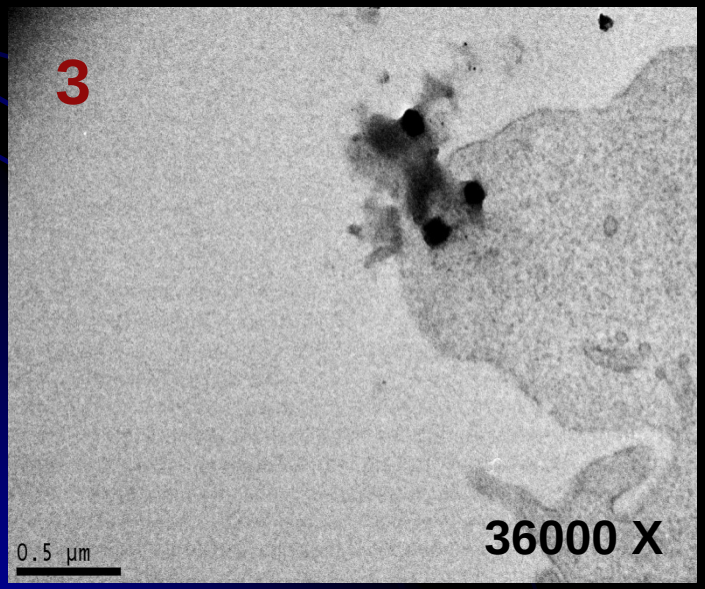


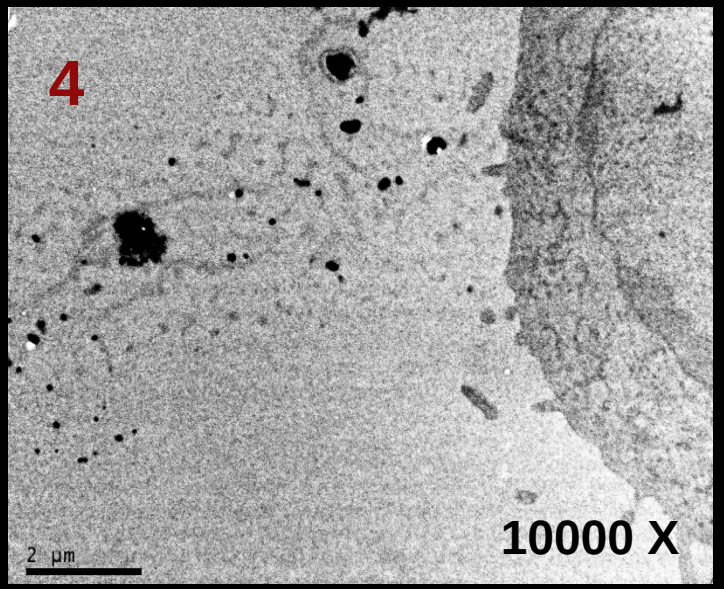


Ni NP Treated A549 Cells

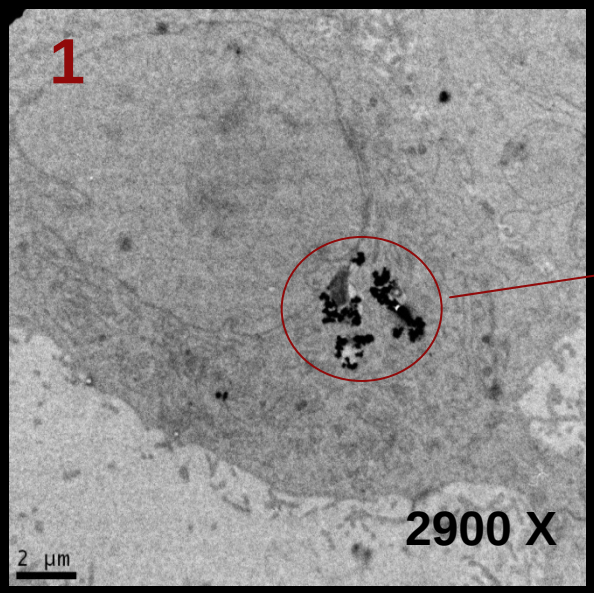


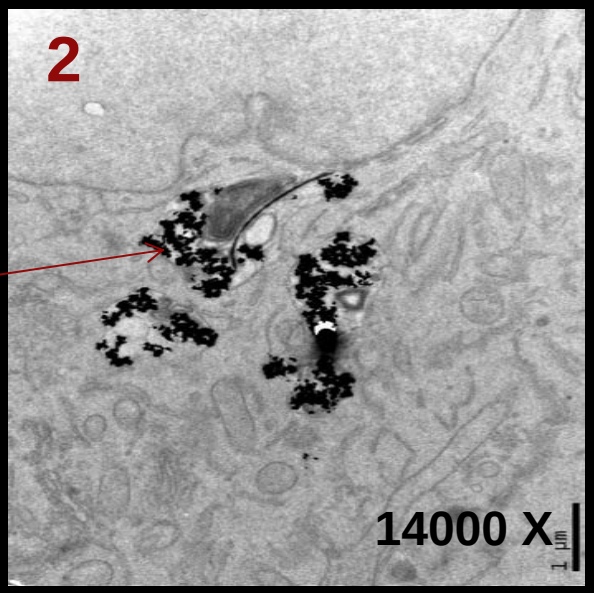


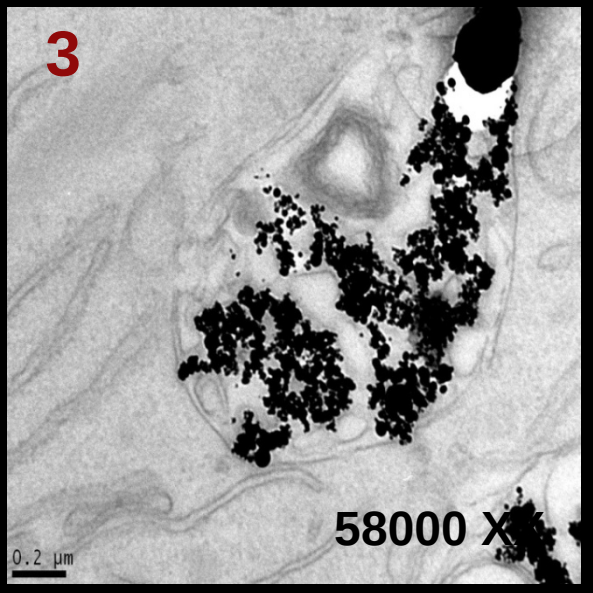


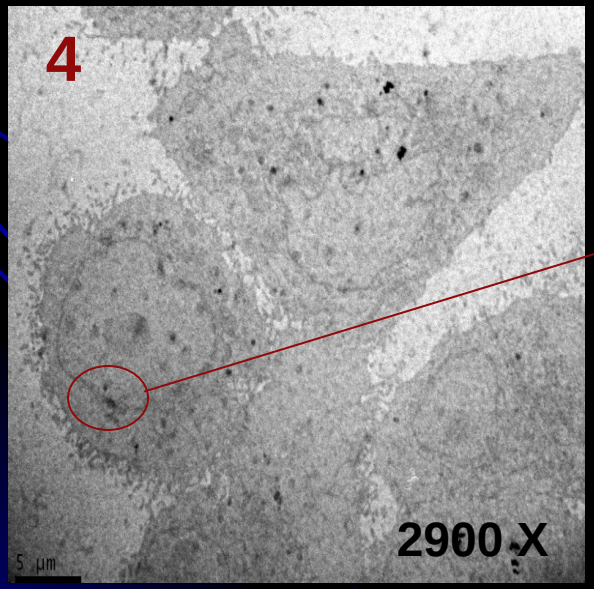


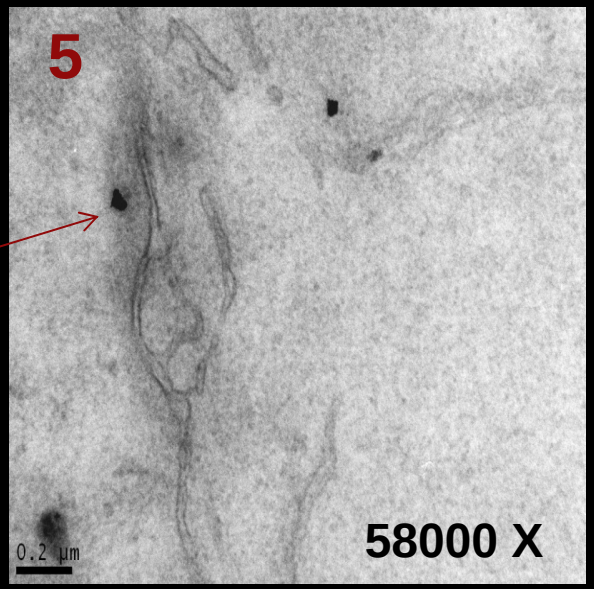
C/Ni NP treated A549 cells











Summary - MNPs Set 2

NP Type / Toxicity Measure	% Mitochondrial Function compared to control#	% Membrane Integrity compared to control#	Cell Uptake (ICP-MS) ng Cu/million cells*	Cell Uptake (TEM) *
Ni	94.8 ± 11.0	86.7 ± 4.0	NA	-
C/Ni	63.4 ± 11.6	101.7 ± 7.0	NA	+
Cu	81.7 ± 5.8	62.9 ± 9.0	122.4 ± 24.3	+
C/Cu	55.9 ± 5.5	62.4 ± 8.0	1016.6± 145.7	+

* ICP MS and TEM analysis,10 µg/ml, 8 hr

MTT and Membrane integrity analysis,10 µg/ml, 24 hr

Largely Different from control

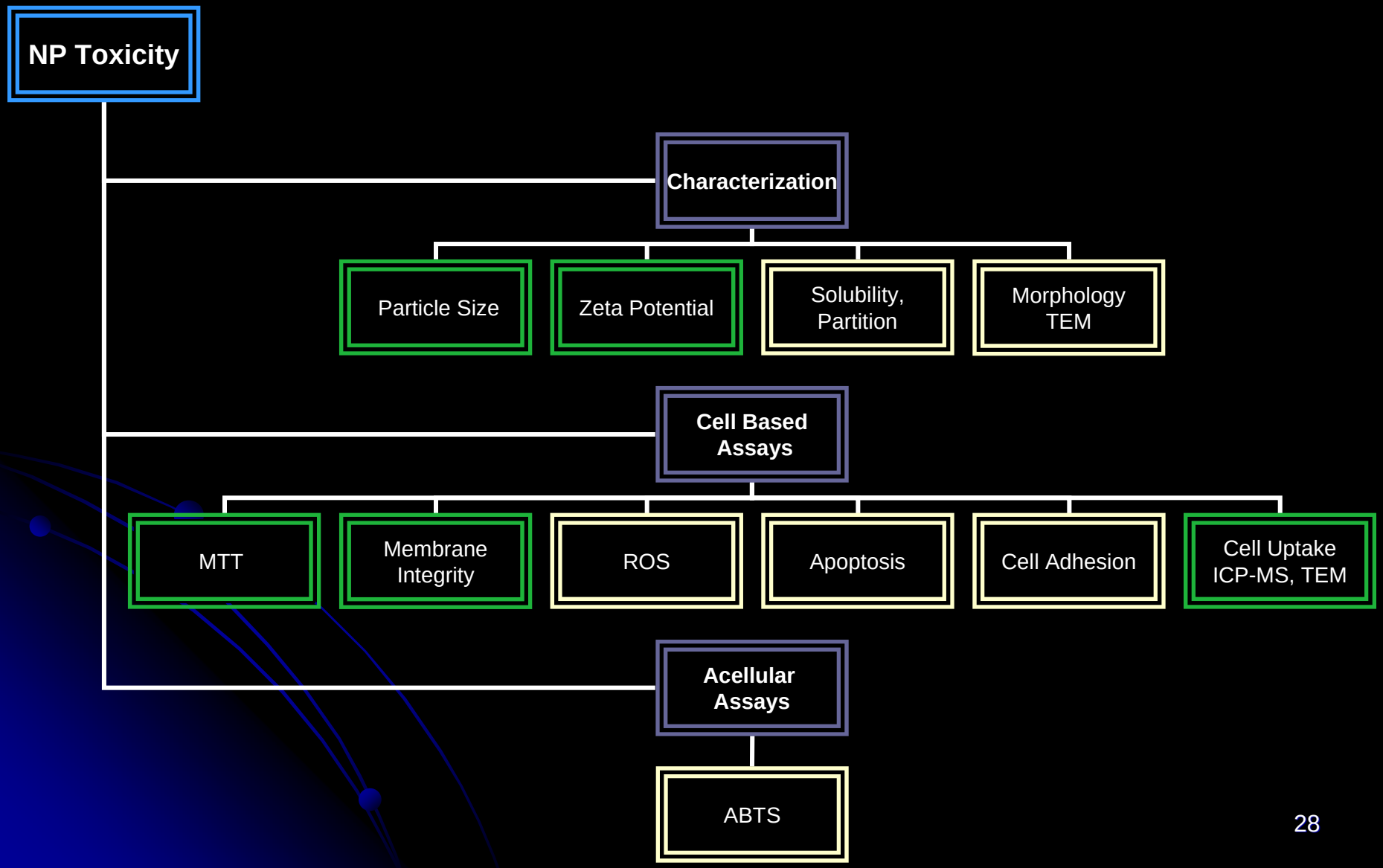
Slightly Different from control

Similar to control

Conclusions - MNPs Set 2

- Average particle size measured by DLS of all MNPs in Set 2 are on an average 20-fold higher than provided by manufacturer.
- Ni NPs do not alter mitochondrial function and membrane integrity and this correlates with the TEM data.
- C/Ni NPs alter the mitochondrial function but not membrane integrity.
- Cu NPs alter mitochondrial function at 100 µg/ml but can alter membrane integrity even at 10 µg/ml dose.
- Rounded morphology of Cu NP treated cells and results from ICP-MS suggest that Cu NPs might act on cell surface at lower dose, possibility of alterations with cell adhesion.
- C/Cu NPs alter mitochondrial function and membrane integrity to the same extent.
- Cu and C/Cu NPs appear to be more toxic than Ni and C/Ni NPs.

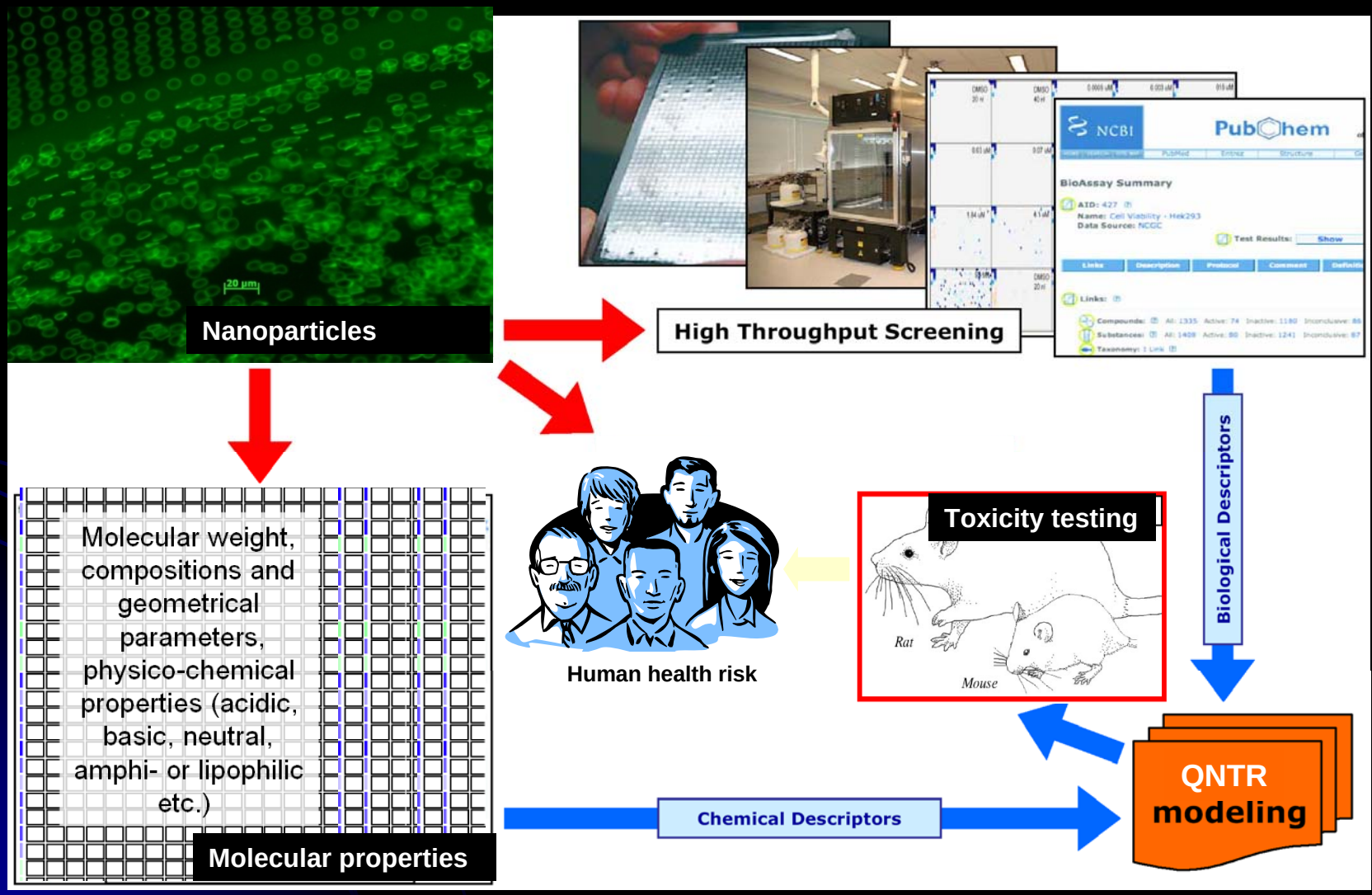
Future Studies



Future Direction

Carolina Exploratory Center for Cheminformatics Research
Accelerating Chemical Genomics Research by Cheminformatics
Alexander Tropsha, Ph.D., Director

Predictive (Computer aided) nano-toxicology



Acknowledgement

- Dr. Russell Mumper
- UNC Chemistry: Dr. Habibi
- UNC CHANL: Ambrose Wallace
- SRC/SEMATECH ERC for funding