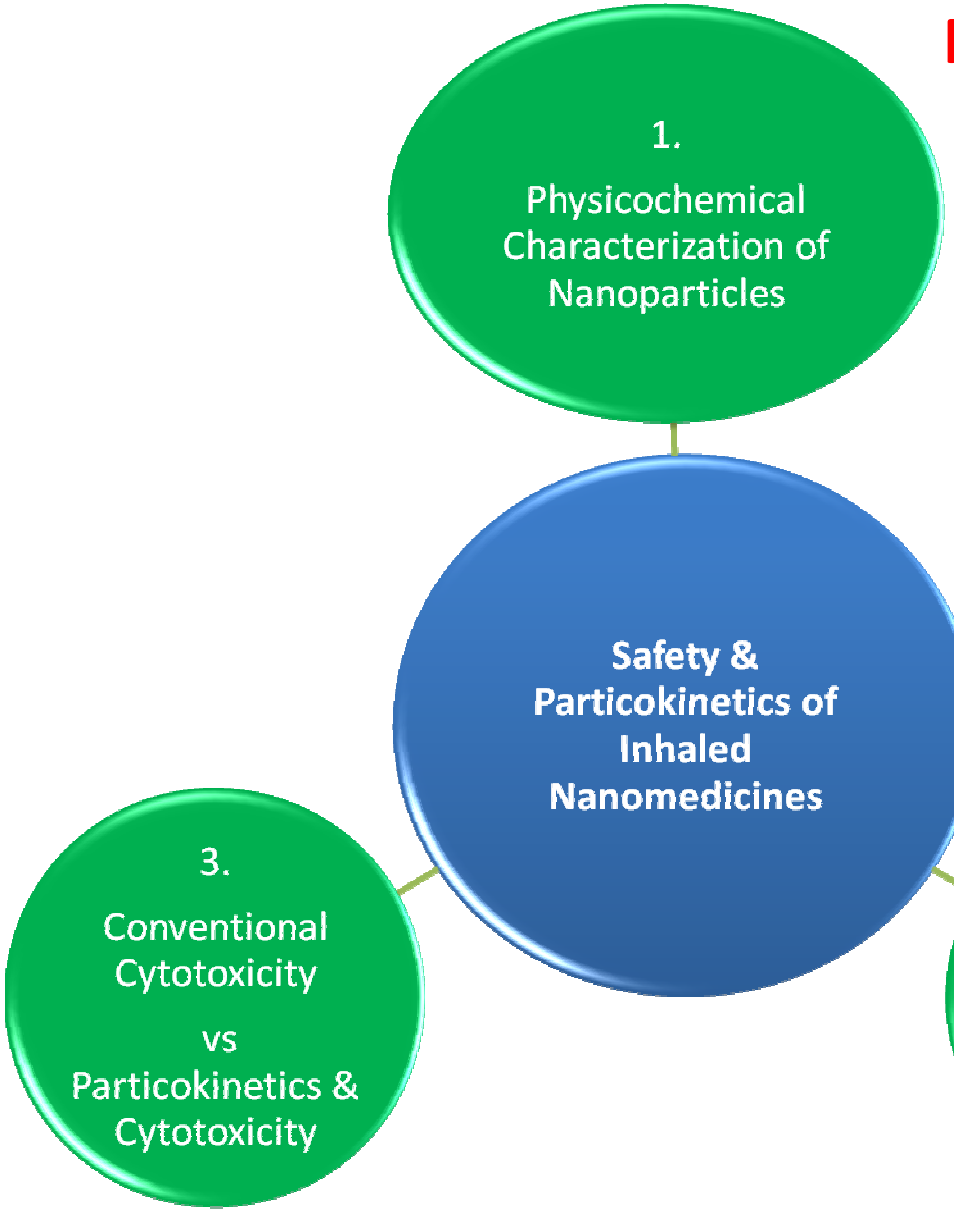
Dosimetry *in vitro* – an under appreciated problem?

ABHINAV KUMAR

Nanomedicine Market to Cross $160 Billion by 2015

Global Market for Nanomedicine –
Global Industry Analysts Inc.



Challenges

1.Toxicity issues with nanomaterials

2.Issues with scaling up production

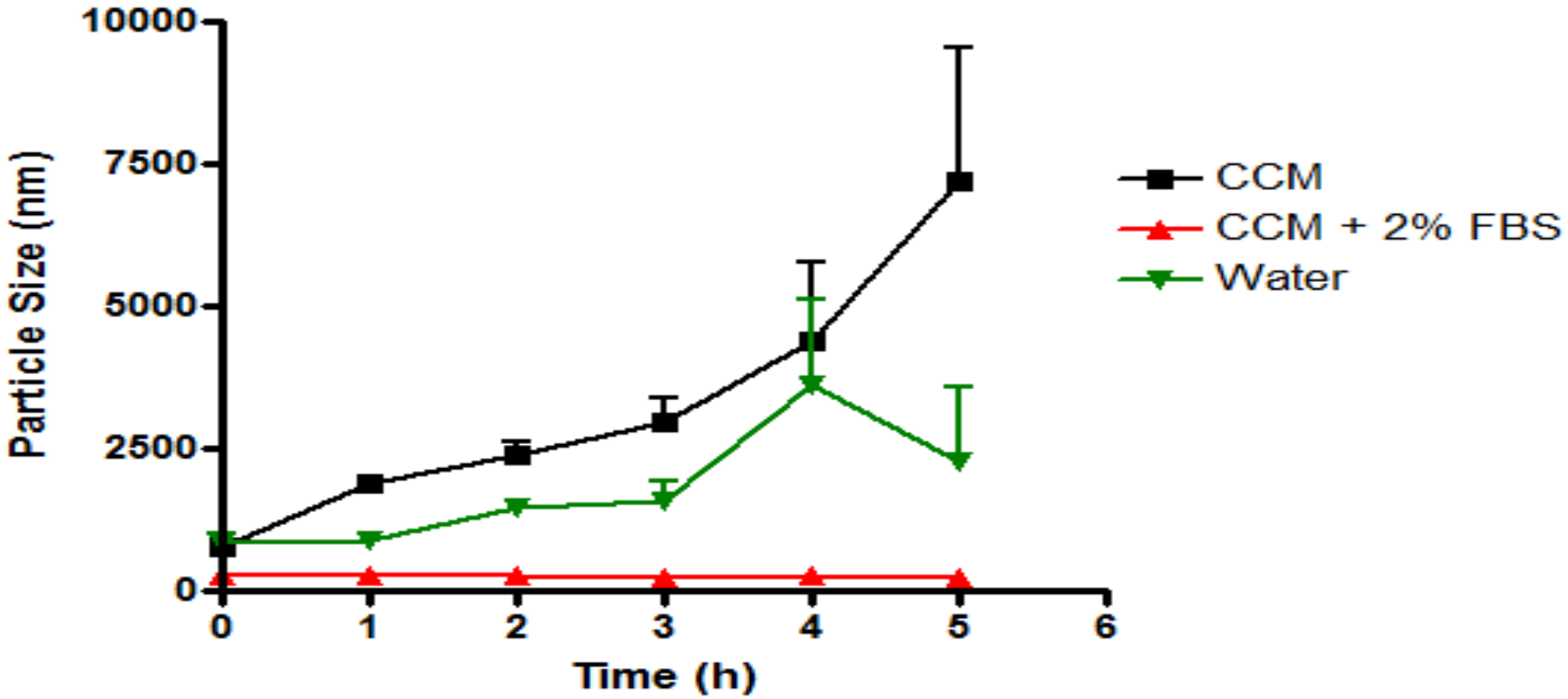
3.Lack of regulatory framework

Limitations of Existing Systems

- Insufficient particle characterization prior to toxicity testing
- Deficiencies in assay design (selection of cell types, time of exposure and dose range)
- Lack of consideration of particle kinetics (dosimetry)

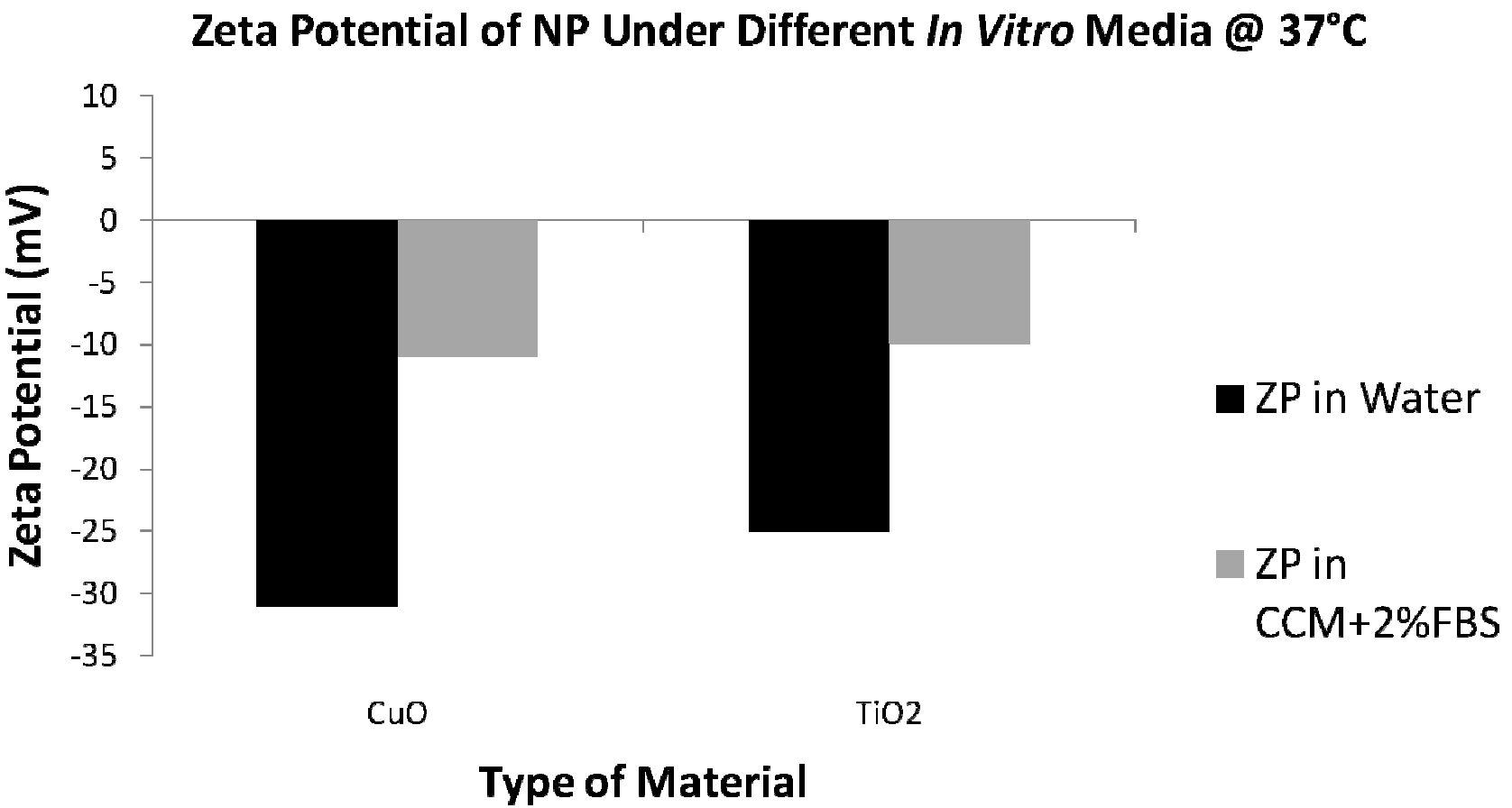
Particle Characterization In Relevant Assay Medium

CuO



Particles aggregate in CCM without FBS and aggregation is a gradual process

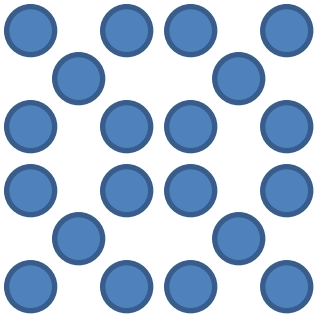
Zeta Potential is Affected by Suspension Media



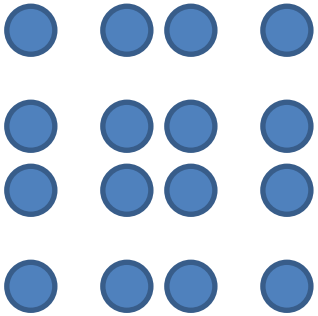
Particles have roughly same ZP in CCM + 2%FBS

Nanoparticles Oxidative Potential Using Ascorbic Acid Depletion Assay

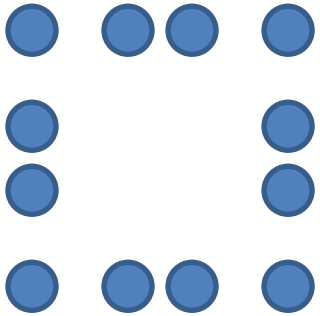
Absorbance measured at 265 nm



t=0
Ascorbic acid
absorption
A1



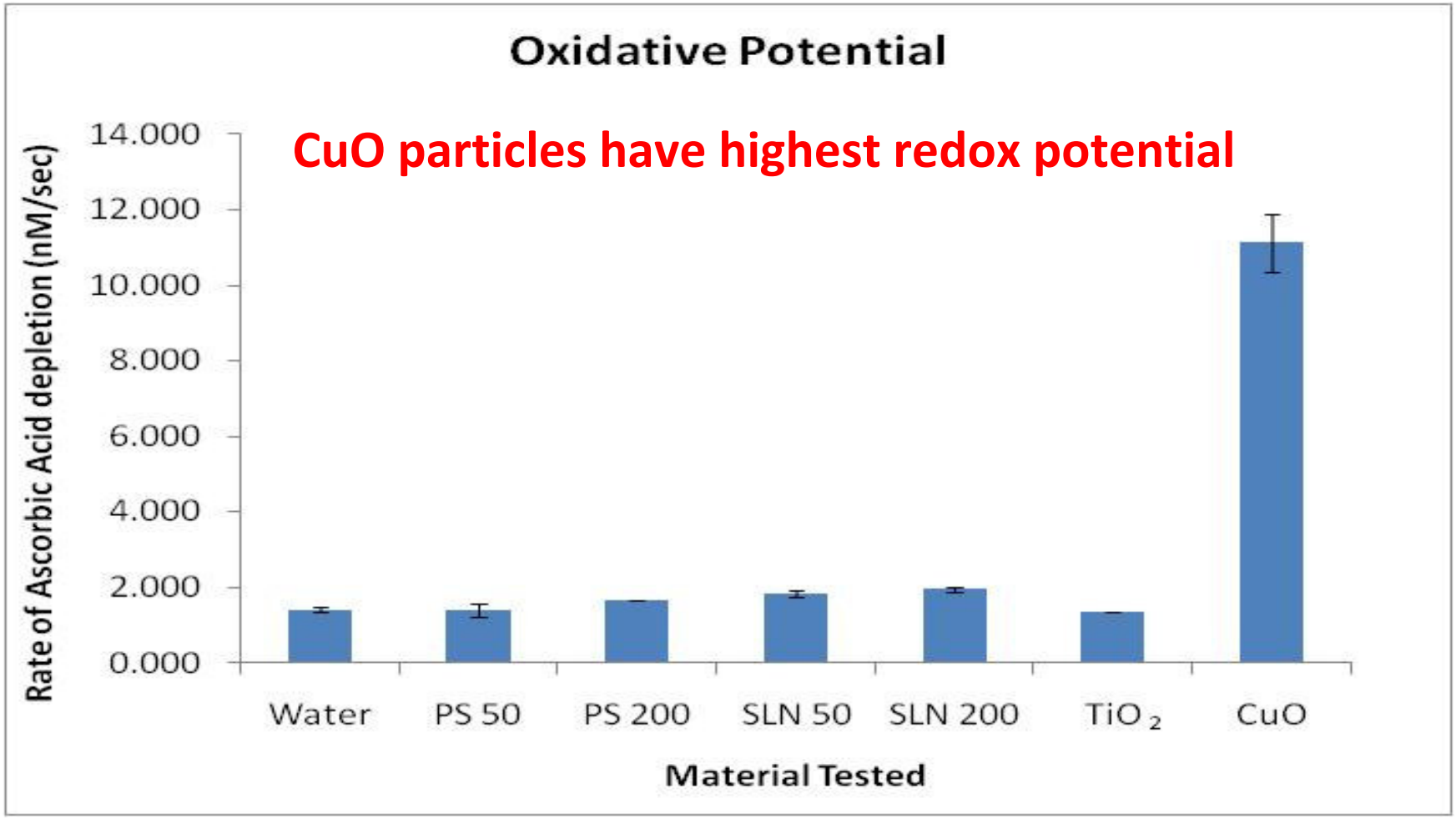
t=2
Ascorbic acid
absorption
A2



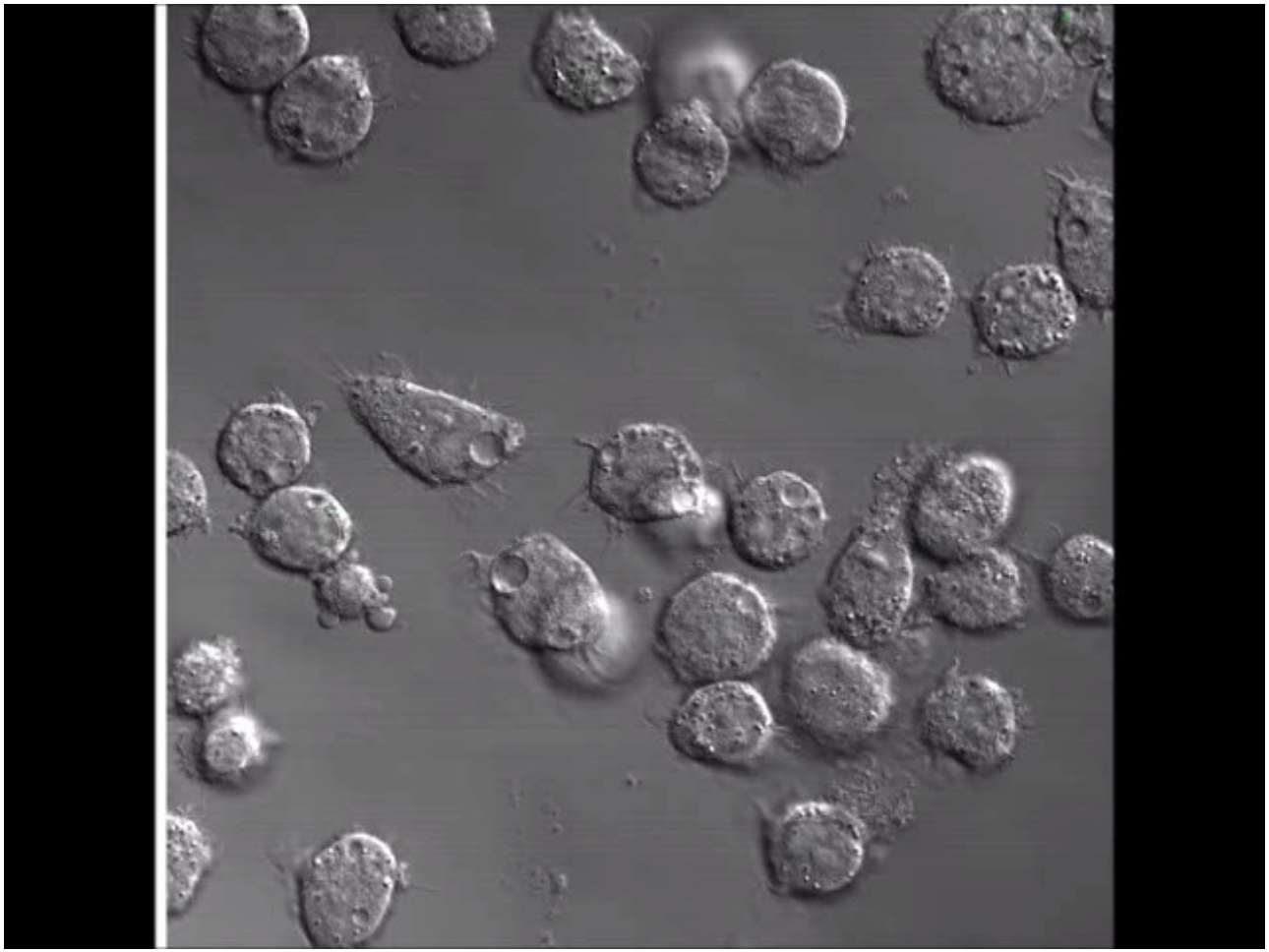
t=4
Ascorbic acid
absorption
A3

A1>A2>A3…… Absorbance of ascorbic acid measured every 2 min for 120 min

Manufacturing Process Does Not Contaminate SLN's Surface

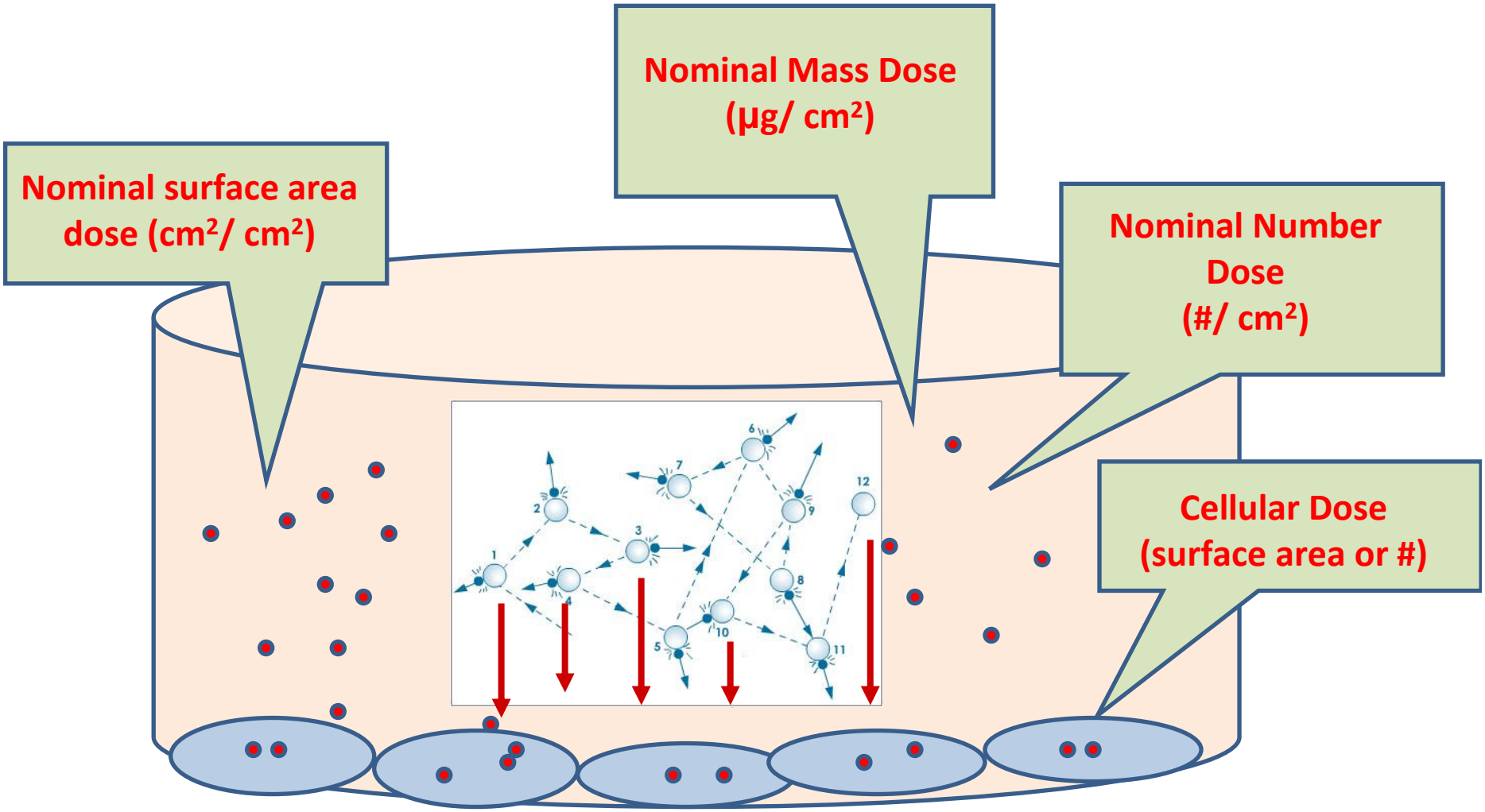


Dosimetry: Do All Particles Reach Cells?



Clift *et. al.* 2008

Dosimetry: Do All Particles Reach Cells?



How Do We Model Cellular Dose?

Gravitational sedimentation can be determined using Stoke's Law:

$$\textit{Settling Velocity} = \frac{2r^2 g(\rho_p - \rho_m)}{9\eta}$$

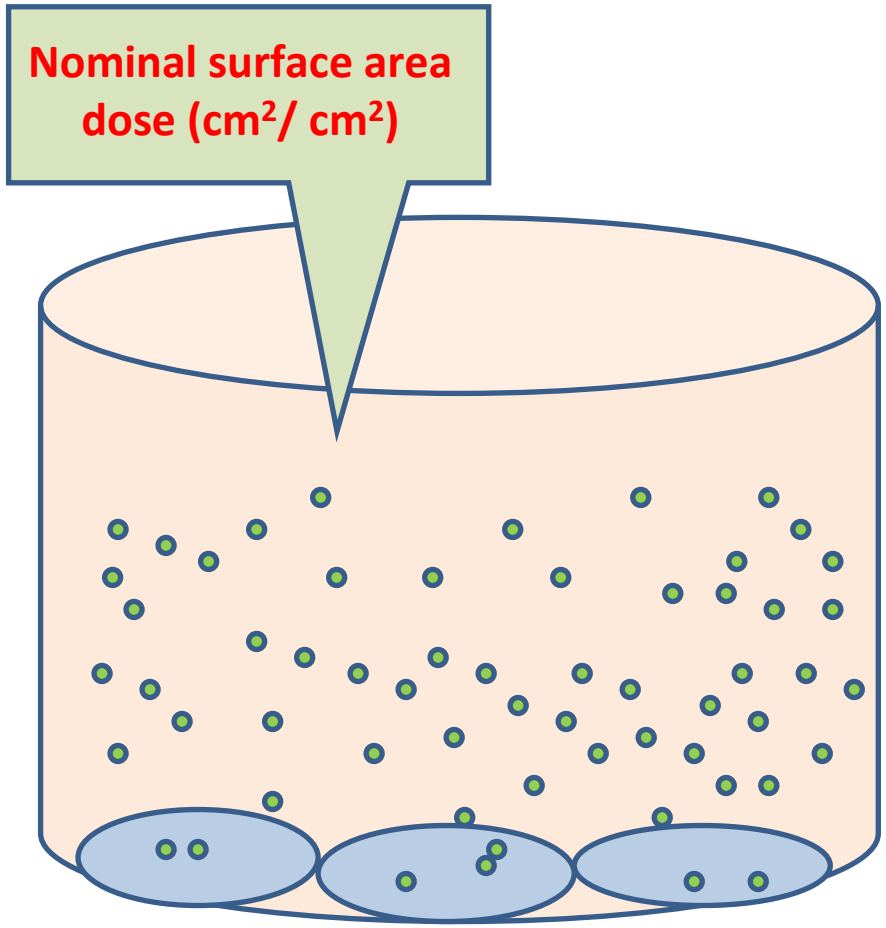
Where
- g is the gravitational acceleration)
- ρ_p is the density of the particle
- ρ_m is the density of the medium
- η is the viscosity of medium
- r, the radius of the particle

Diffusional contribution can be determined using Fick's Law:

$$\frac{\partial c}{\partial t} = \frac{D \partial^2 c}{\partial z^2}$$

Where:
- c is the particle's concentration
- D is the diffusion coefficient
- t is the time
- z is the spatial coordinate (from bottom to top of the culture well)

How Do We Measure Cellular Dose?



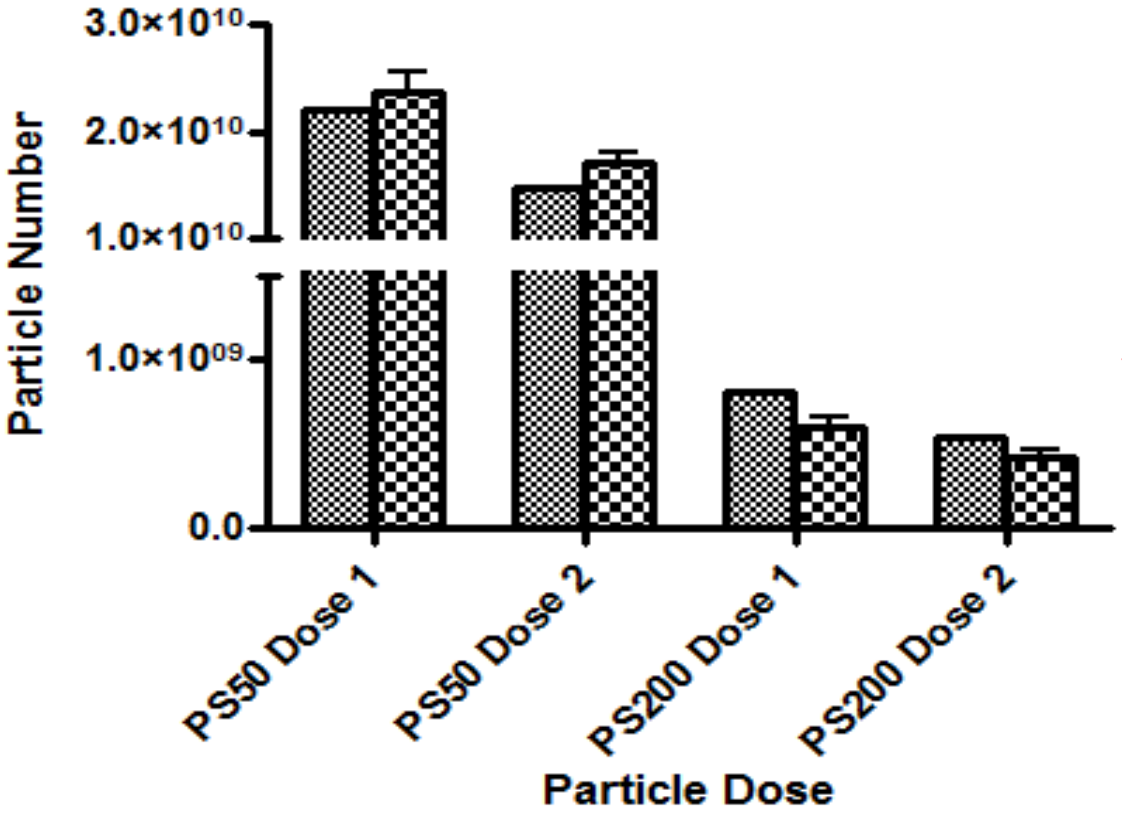
METHOD

Fluorescent PS50 and PS200nm particles were added to cells

After 4 h incubation with particles cells were lysed

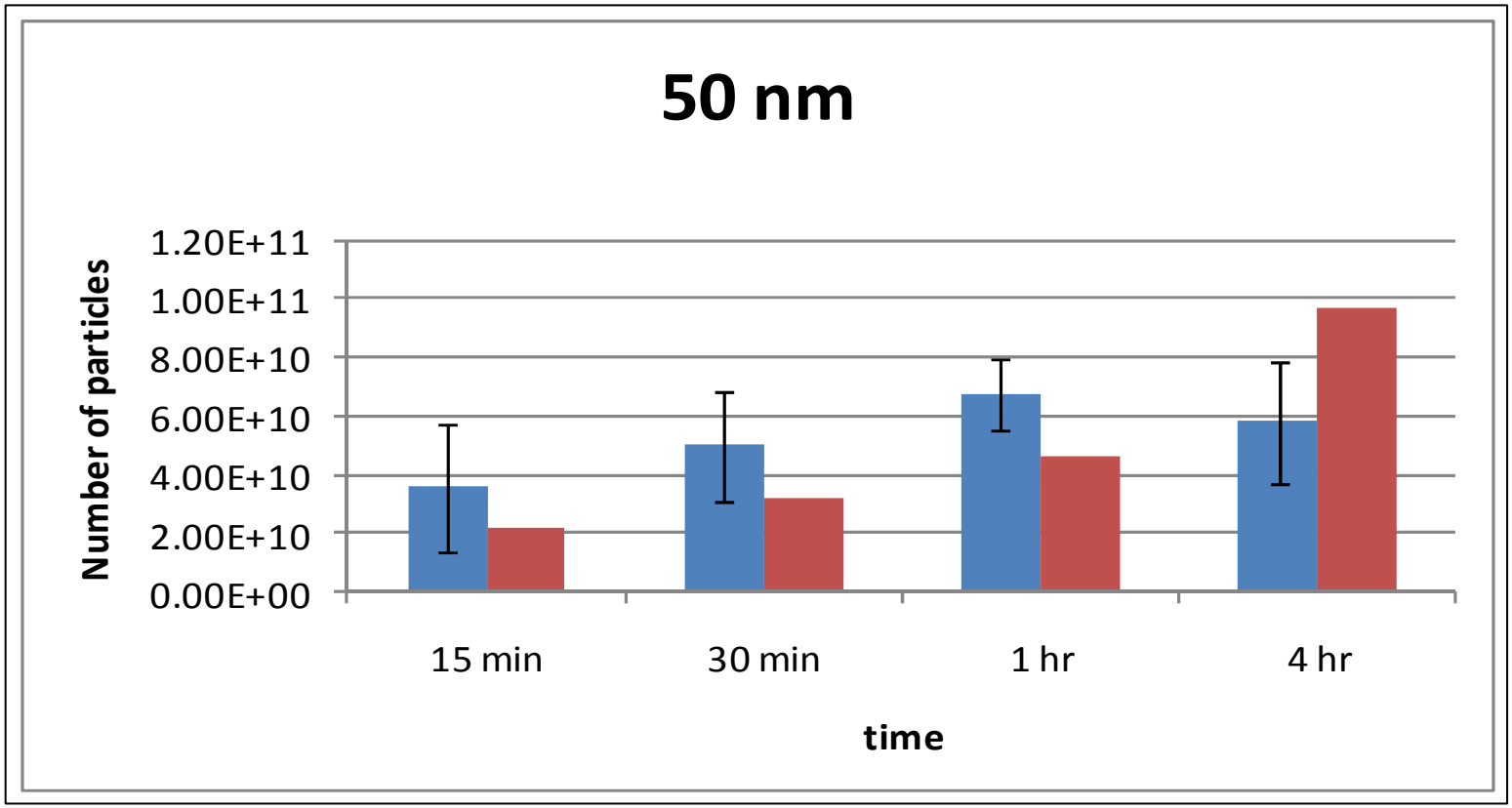
Fluorescence of cell lysate was measured at 469 nm Excitation and 508 nm Emission

Cellular Dose < 20% of Nominal Dose



Predicted dose correlates well with observed dose

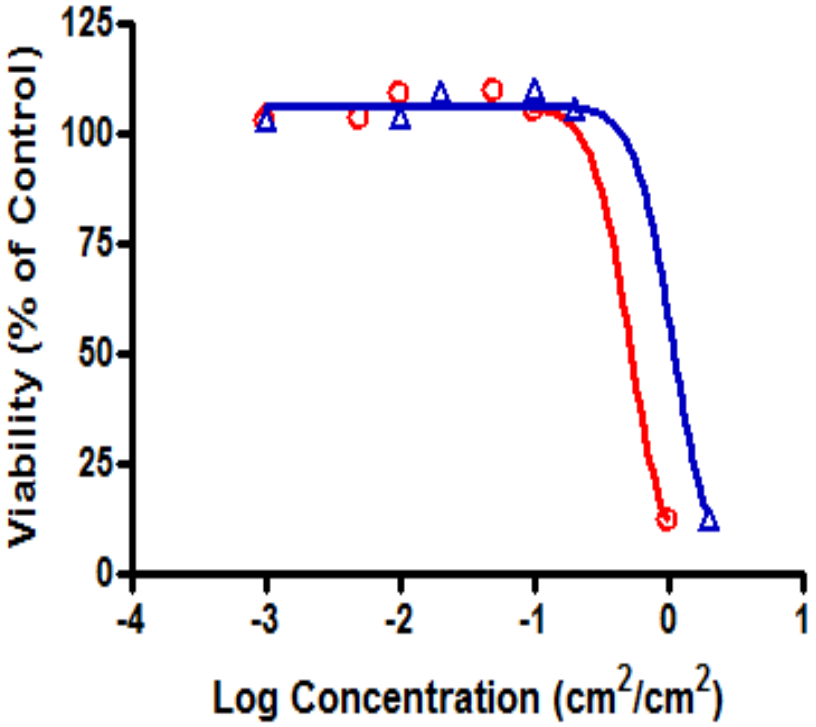
Cellular Dose < 20% of Nominal Dose



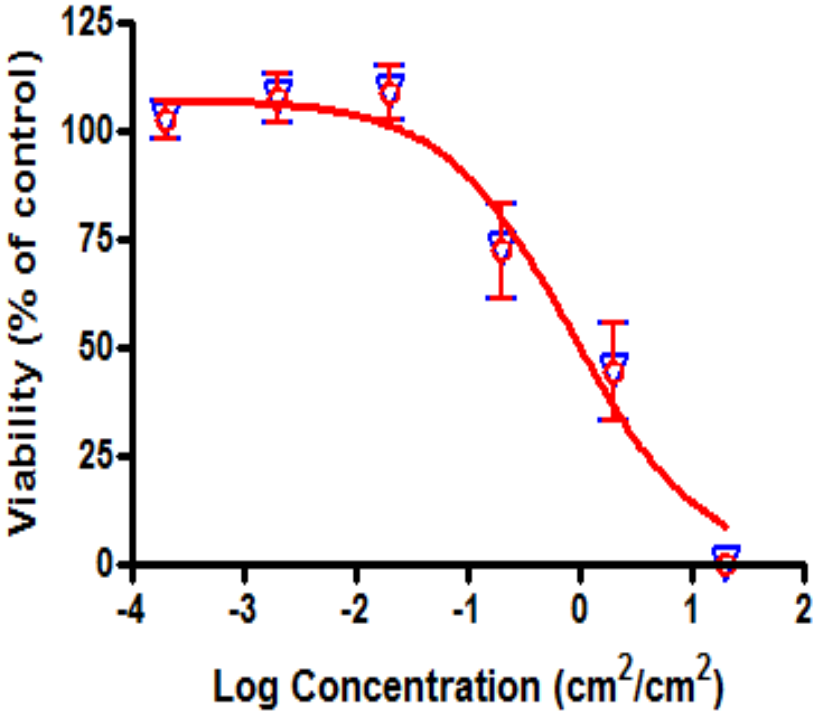
Predicted dose correlates well with observed dose

Nominal Dose Underestimates Cytotoxicity of CuO

6 h



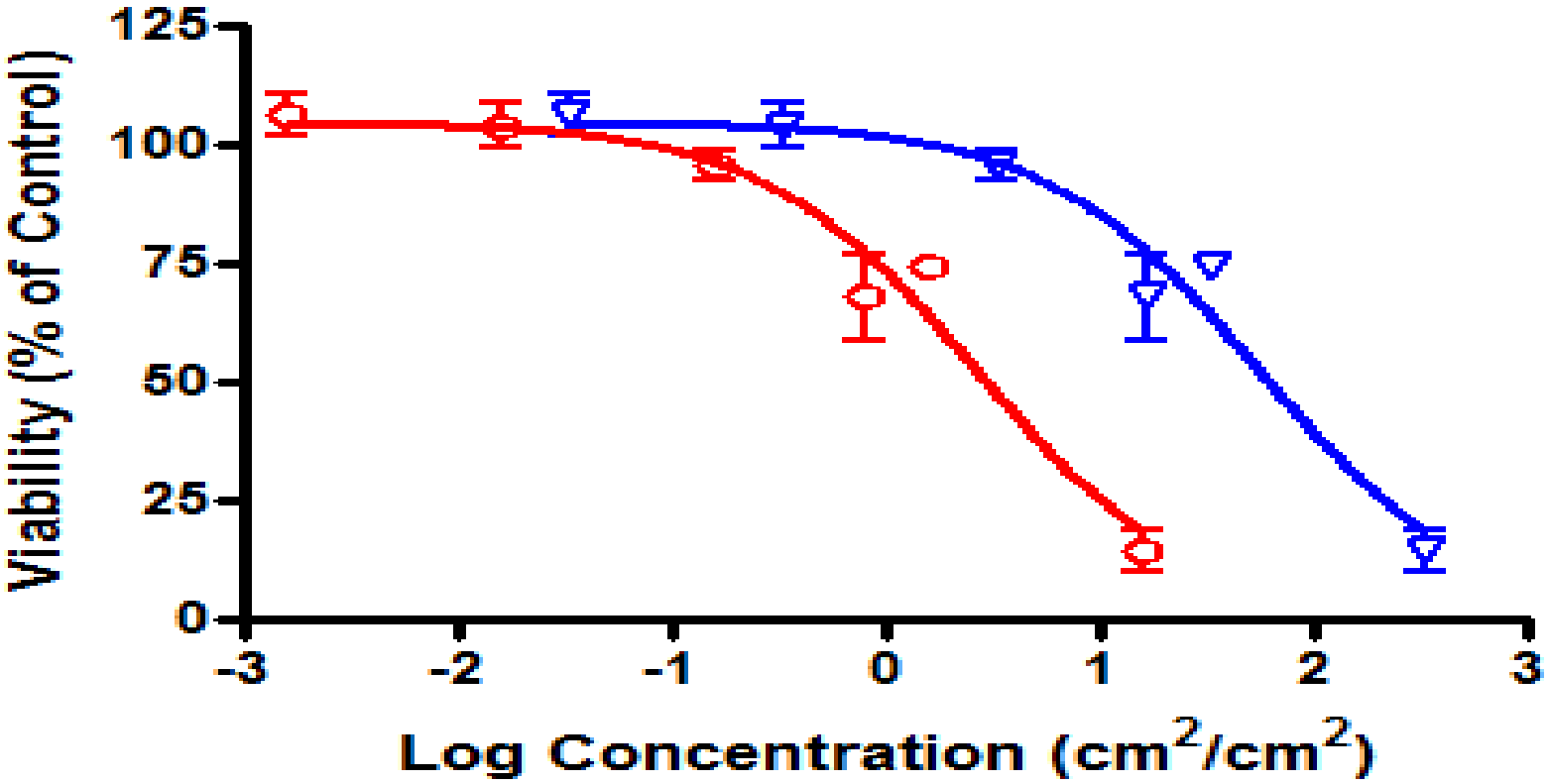
24 h



▽ Nominal SA Dose (cm^2/cm^2)

○ Cellular SA Dose (cm^2/cm^2)

Nominal Dose Underestimates Cytotoxicity of LNC 50

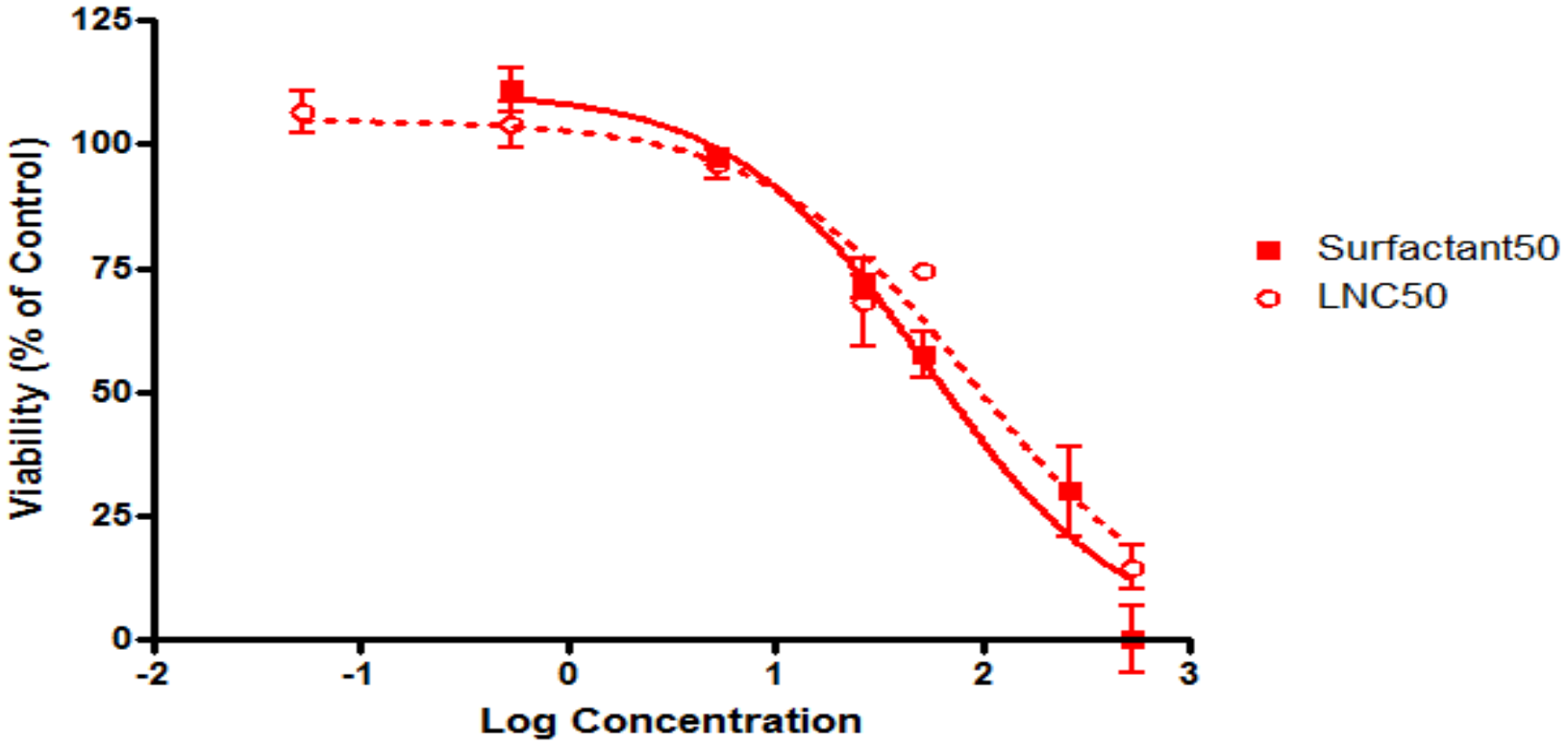


▽ Nominal SA Dose (cm² /cm²) ○Cellular SA Dose (cm² /cm²)

Nominal Dose Underestimates Cytotoxicity

Cell Type	Particle Type	NOMINAL SA DOSE	CELLULAR SA DOSE
		IC_{50} (cm^2/cm^2)	IC_{50} (cm^2/cm^2)
A549	CuO 6 h	1	0.5
	CuO 24 h	0.8	0.8
	LNC 50	55	3

Toxicity Associated Is Due To Solutol



In Vitro-In Vivo Correlation

Assessing Toxicity of Fine and Nanoparticles: Comparing *In Vitro* Measurements to *In Vivo* Pulmonary Toxicity Profiles

Christie M. Sayes, Kenneth L. Reed, and David B. Warheit[1]

DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware 19714

When considering the range of toxicity end points to five different particle types, the comparisons of ***in vivo*** and ***in vitro*** measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study—such as cell types to be utilized, culture conditions and time course of exposure, as well as measured end points. It seems clear that ***in vitro*** cellular systems will need to be further developed, standardized, and validated (relative to ***in vivo*** effects) in order to provide useful screening data on the relative toxicity of inhaled particle types.

What Are The Implications?

Particle Characterization

- Size Is Affected By Suspension Media
- Aggregation Is A Gradual Process

Dosimetry

- Way of Expressing Dose Can Skew The Interpretation of Results
- Cellular SA Dose Should Be Used To Express *In Vitro* Results

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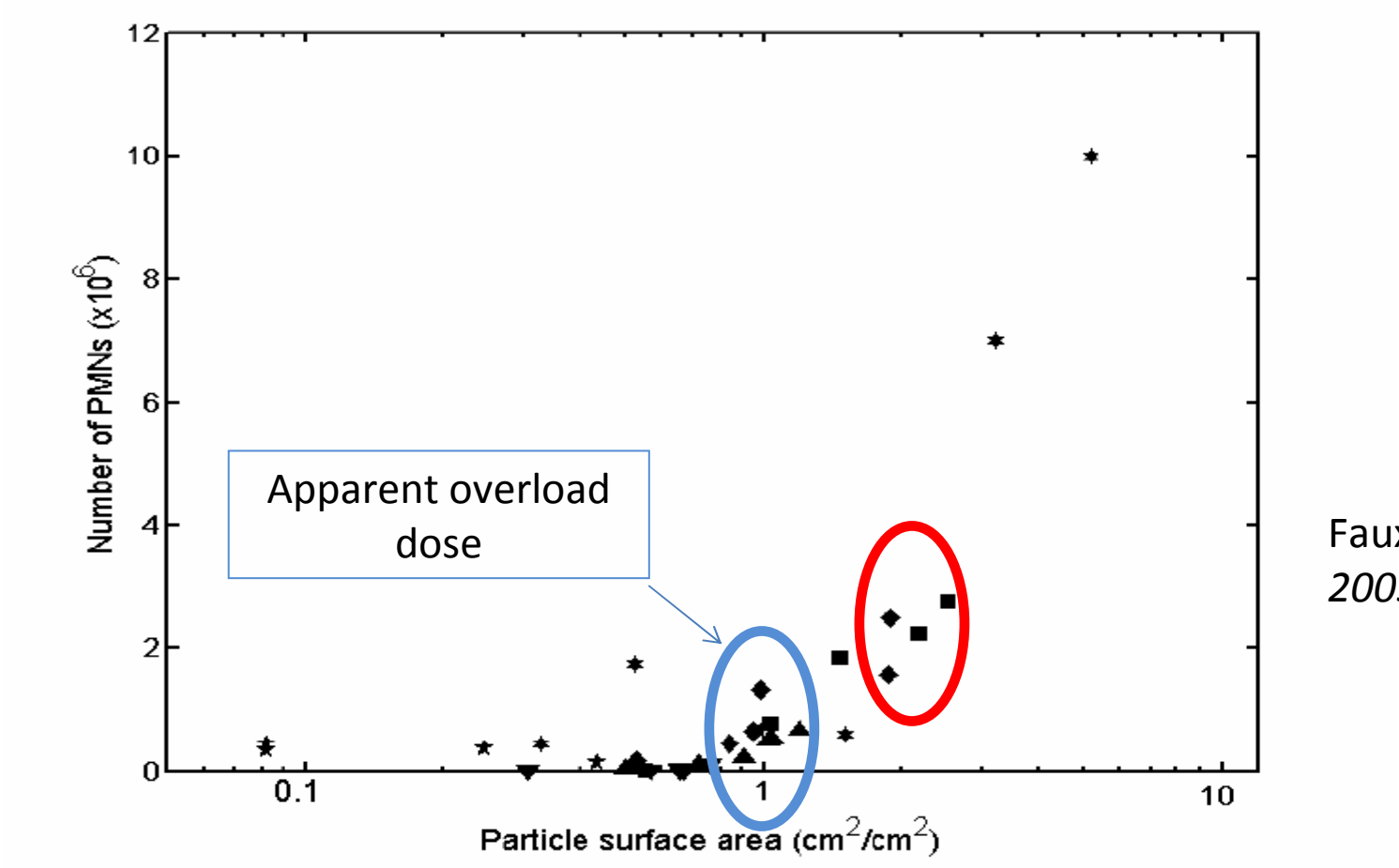
- Thanks to all the project students

KING'S College LONDON

Unilever

Thank You

Threshold Dose *In Vivo* Results



Faux *et al., 2003*

The dose-response *in vivo* using normalised dose (cm^2/cm^2).