NATIONAL NANOTECHNOLOGY INITIATIVE

The National Nanotechnology Initiative presents

A Joint US-EU Workshop

March 10 - 11, 2011

Washington, DC

In Vitro – In Vivo Correlations of Dose- and Response-Metrics: Concepts for OEL Extrapolation

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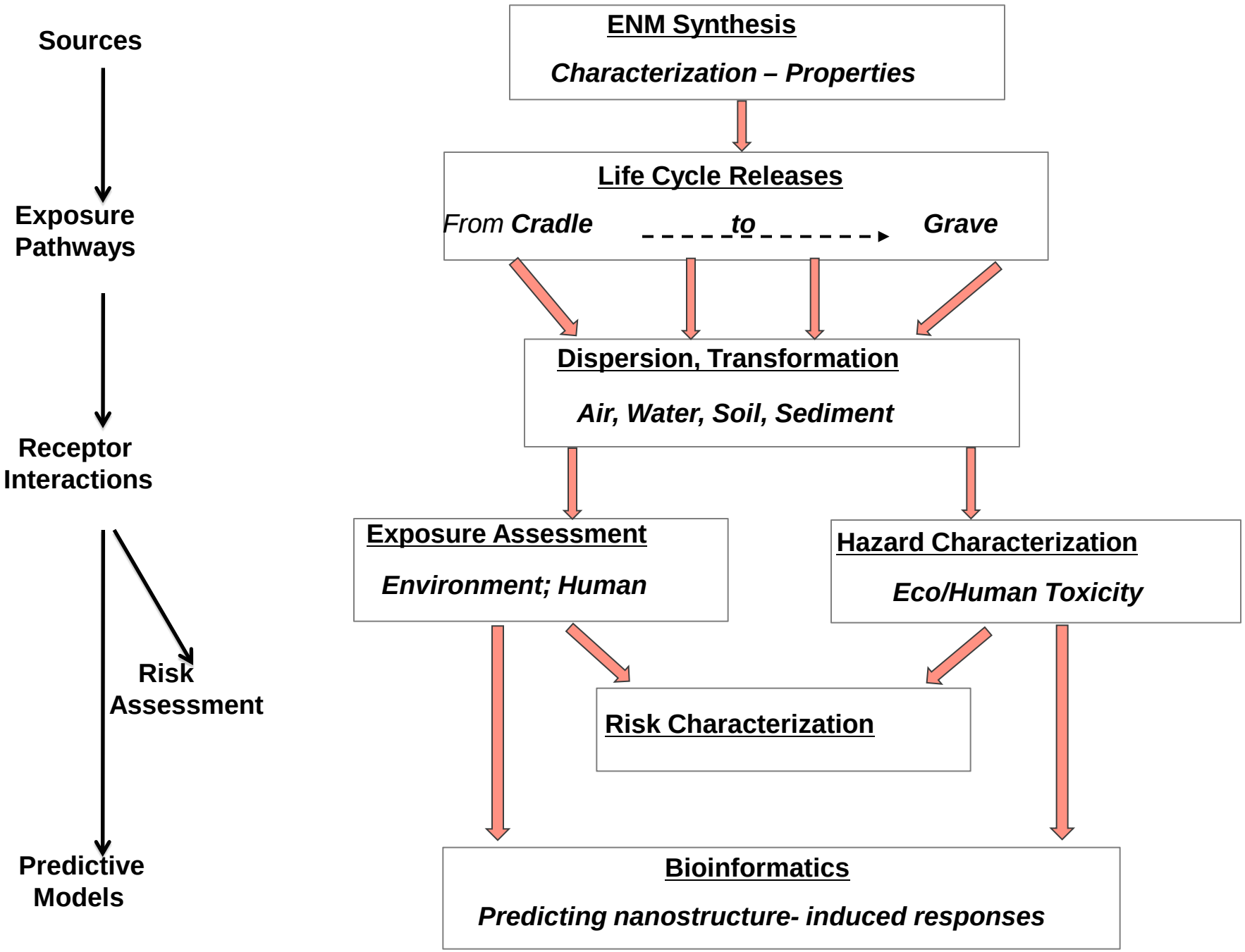
March 11, 2011

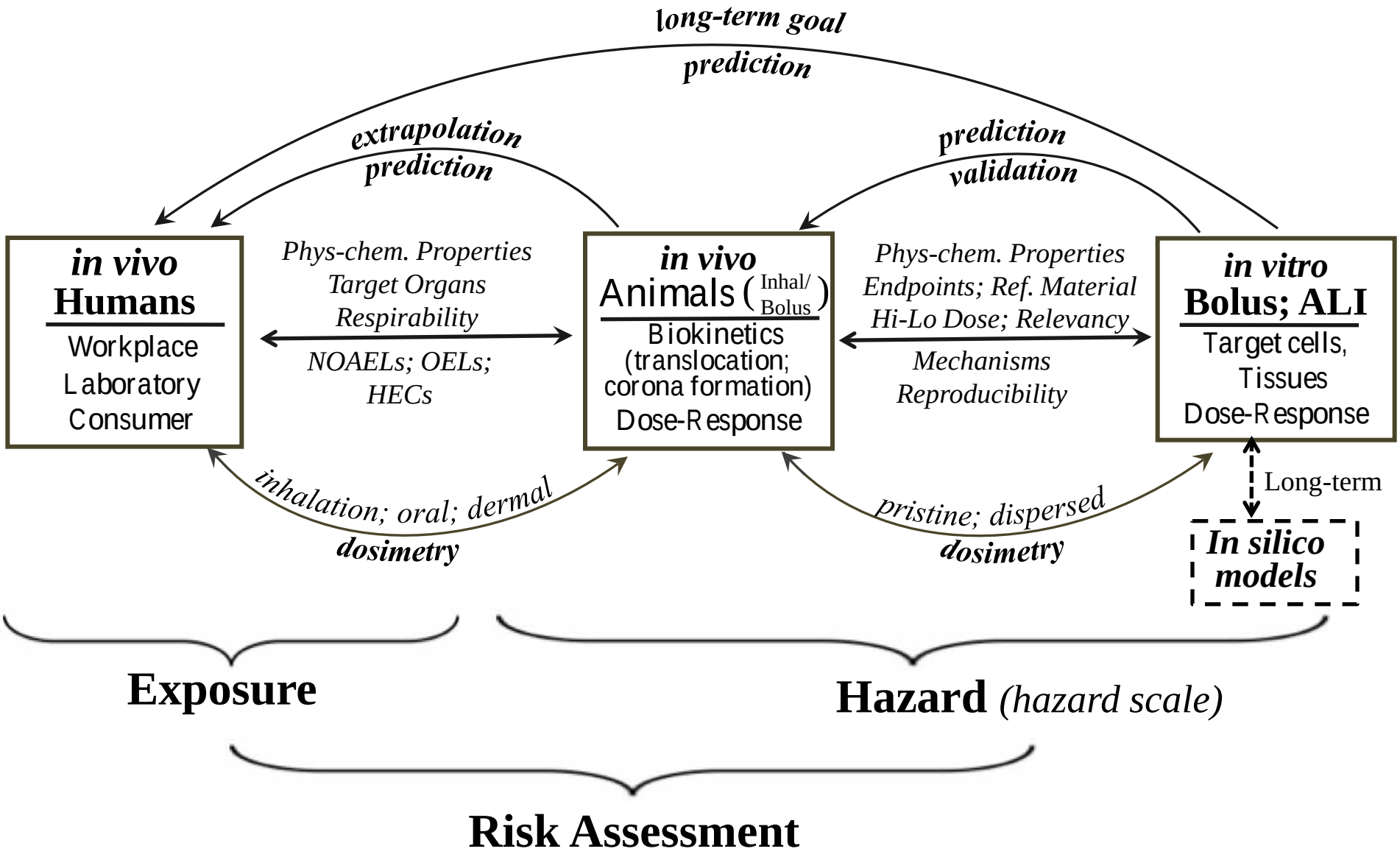
UNIVERSITY of ROCHESTER MEDICAL CENTER

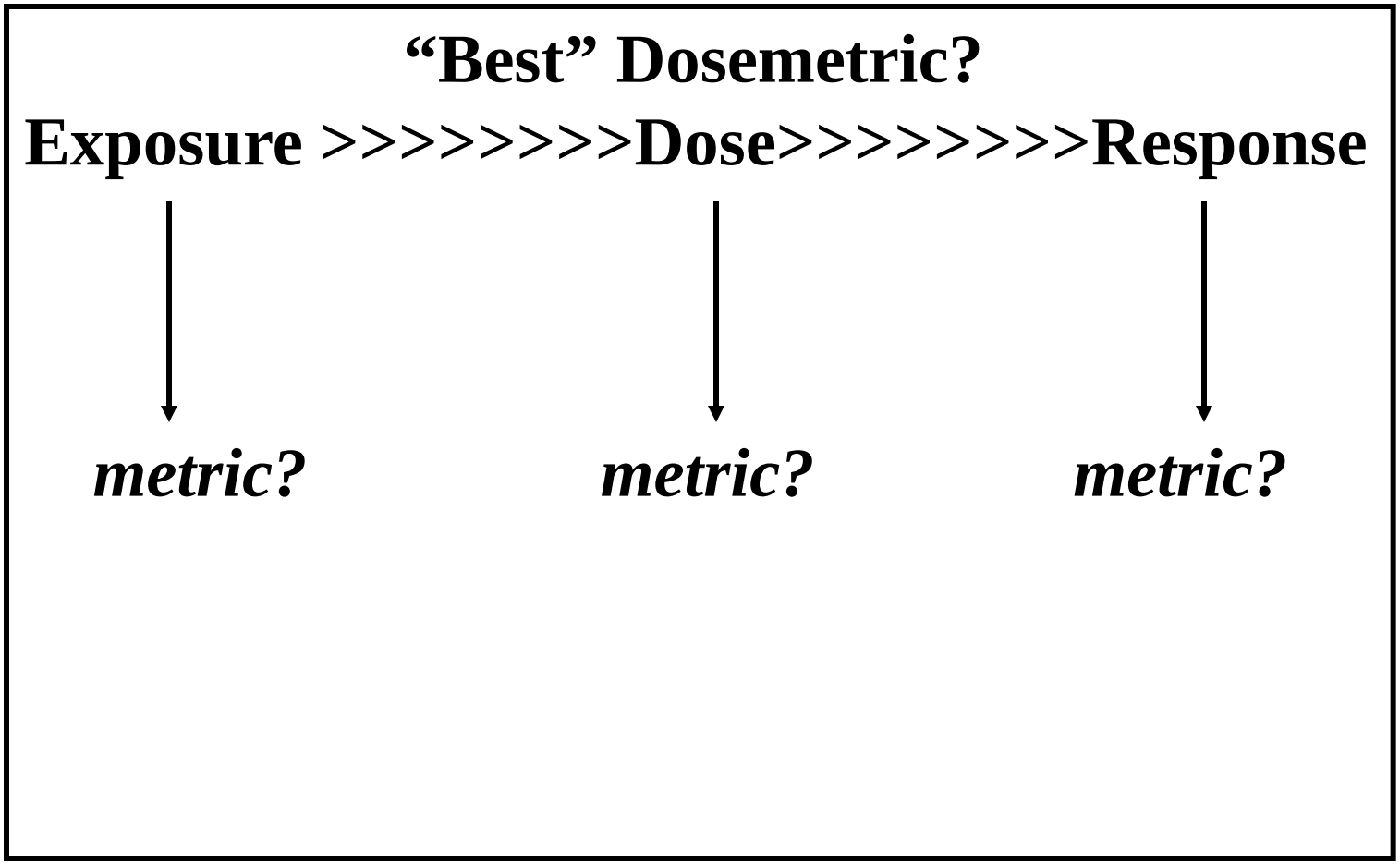
Data Needs for ENM Induced Environmental and Health Effects and Associated Risks



Concepts of Nanomaterial Toxicity Testing:

Risk Assessment as Function of Exposure and Hazard




“Best” Dosemetric?
Exposure >>>>>>>>>Dose>>>>>>>>>Response
metric?
metric?
metric?

Mass/Number/surface Area Correlations for Selected NPs

Particle Type	Diameter nm	Density g/cm^3	Spc. Srf. Area m^2/g	Specific # number/g	Airborne Conc. of 100 µg/m^3	
					Surface cm^2/m^3	Number $\#/m^3$
Pt	50	21.09	5.69	$7.24x10^{14}$	5.69	$7.24x10^{10}$
Gold	50	19.3	6.22	$7.91x10^{14}$	6.22	$7.92x10^{10}$
Ag	50	7.2	11.43	$1.46x10^{15}$	11.43	$1.46x10^{11}$
Cu	50	8.9	13.48	$1.72x10^{15}$	13.48	$1.72x10^{11}$
Al	50	2.7	44.44	$5.65x10^{15}$	44.44	$5.65x10^{11}$
TiO_2(R)	50	4.23	28.37	$3.61x10^{15}$	28.37	$3.61x10^{11}$
TiO_2(A)	50	3.9	30.77	$3.92x10^{15}$	30.77	$3.92x10^{11}$
C	50	2.26	53.10	$6.76x10^{15}$	53.10	$6.76x10^{11}$
Polystyrene	50	1.05	114.3	$1.46x10^{16}$	114.3	$1.46x10^{12}$

Usual Physical Dose-Metrics for NPs that Correlate with Biol./Toxicol. Effects:

Mass

Number

Surface Area

(Volume)

} ***correlation between these should be part of NP characterization***

BET Surface Area:

Which one?

N; Kr; Ar; others?

Need for standardization: Most common is use of nitrogen.

Most desirable: Measure of bioavailable SA

Also: BET equivalent particle size:

to characterize/estimate agglomeration/aggregation

Physico-Chemical Properties of Investigated Nanoparticles

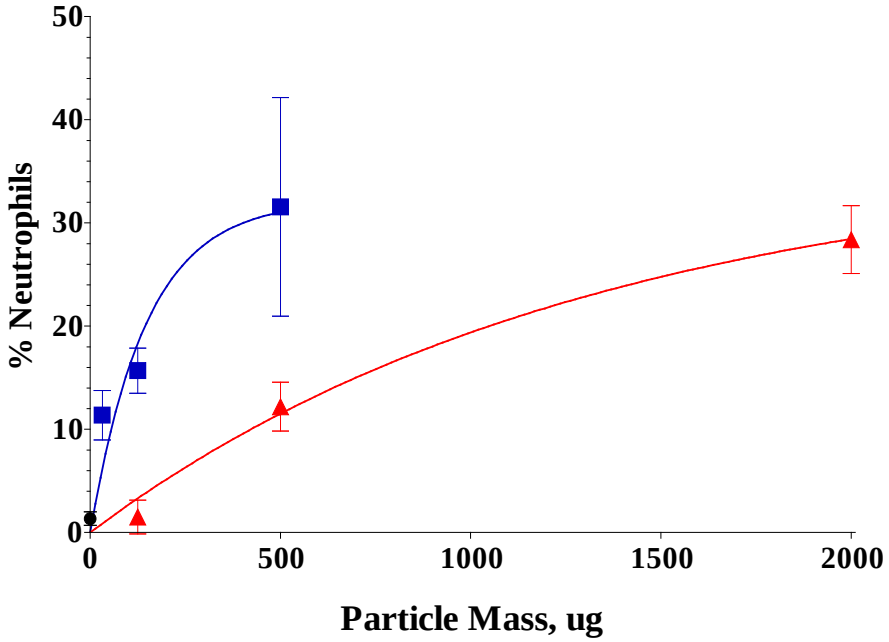
Particle	Origin	Primary Particle Size (nm) *(a)*	Crystal Phase	Specific Surface Area (m^2/gm)	BET Equivalent Diameter (nm)	Hydrodynamic Diameter (nm) *(b)*	Zeta Potential (mV) *(c)*	Agglomm/ Aggregation Degree
Elemental Carbon (EC)	Electric Spark Generated (Rochester)	41	Amorphous	767.9	3.4	204	-50.5	High
TiO_2(F)	Fischer Scientific	250	Anatase	8.0	195	1287	-14.7	Medium
TiO_2(M)	Millenium Chemical Corp.	~20	Anatase	86.1	18.3	1608	14	High
TiO_2(D)	Evonik Chemicals	~25	80% anatase/ 20% rutile	57.4	27	576	27.3	Medium
Copper-40	Nanotechnologies	40	FCC crystal	30.6	21.9	850	-0.6	Medium
Silver-35	Nanotechnologies	35	FCC crystal	21.0	27.3	483	-47.0	Medium
Au_{50}	Ted Pella (Ca)	50	FCC crystal	6	51.8	93	-33.8	Low
PS-NH_3	Bangs Laboratories	65	Amorphous	88	65	72	83.0	Very low

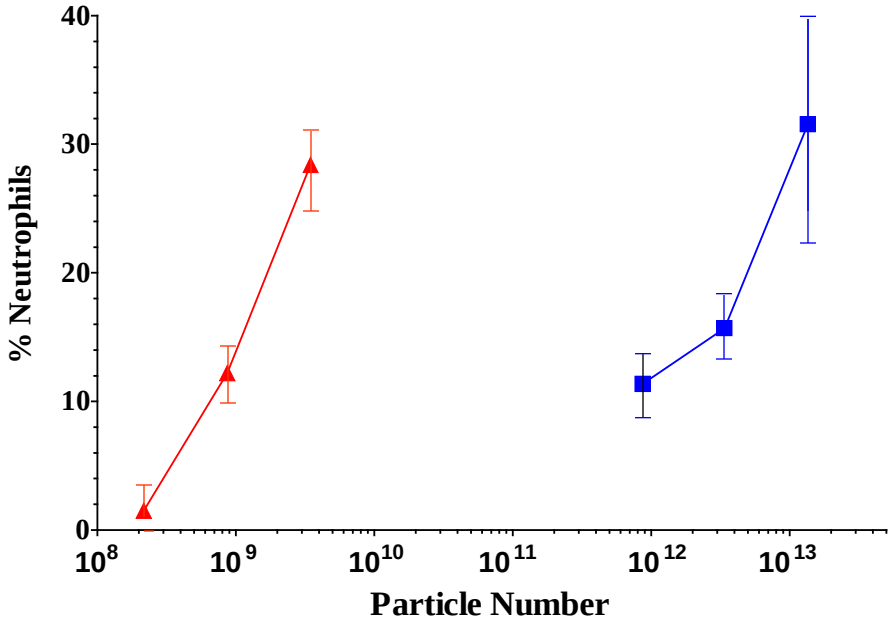
(a) Size as reported by manufacturer; ***(b)*** measured by dynamic light scattering (DLS) in water;
(c) measured by electrophoretic light scattering (ELS) in water.

Which Dose-Metric?

Percent of Neutrophils in Lung Lavage 24 hrs after Intratrachael Dosing of Ultrafine and Fine TiO_2 in Rats

fine TiO_2 (200nm)
ultrafine TiO_2 (25nm)
saline



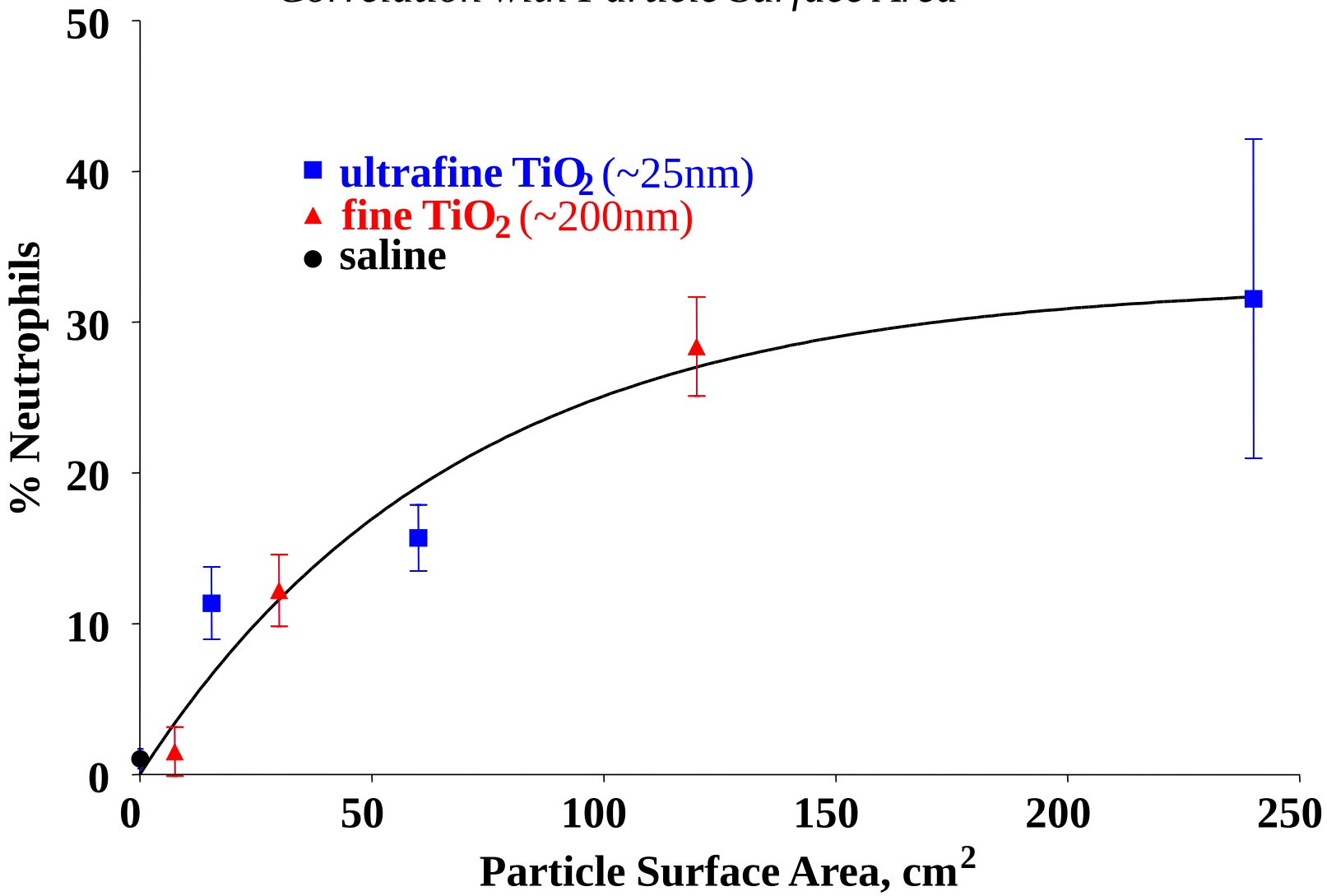


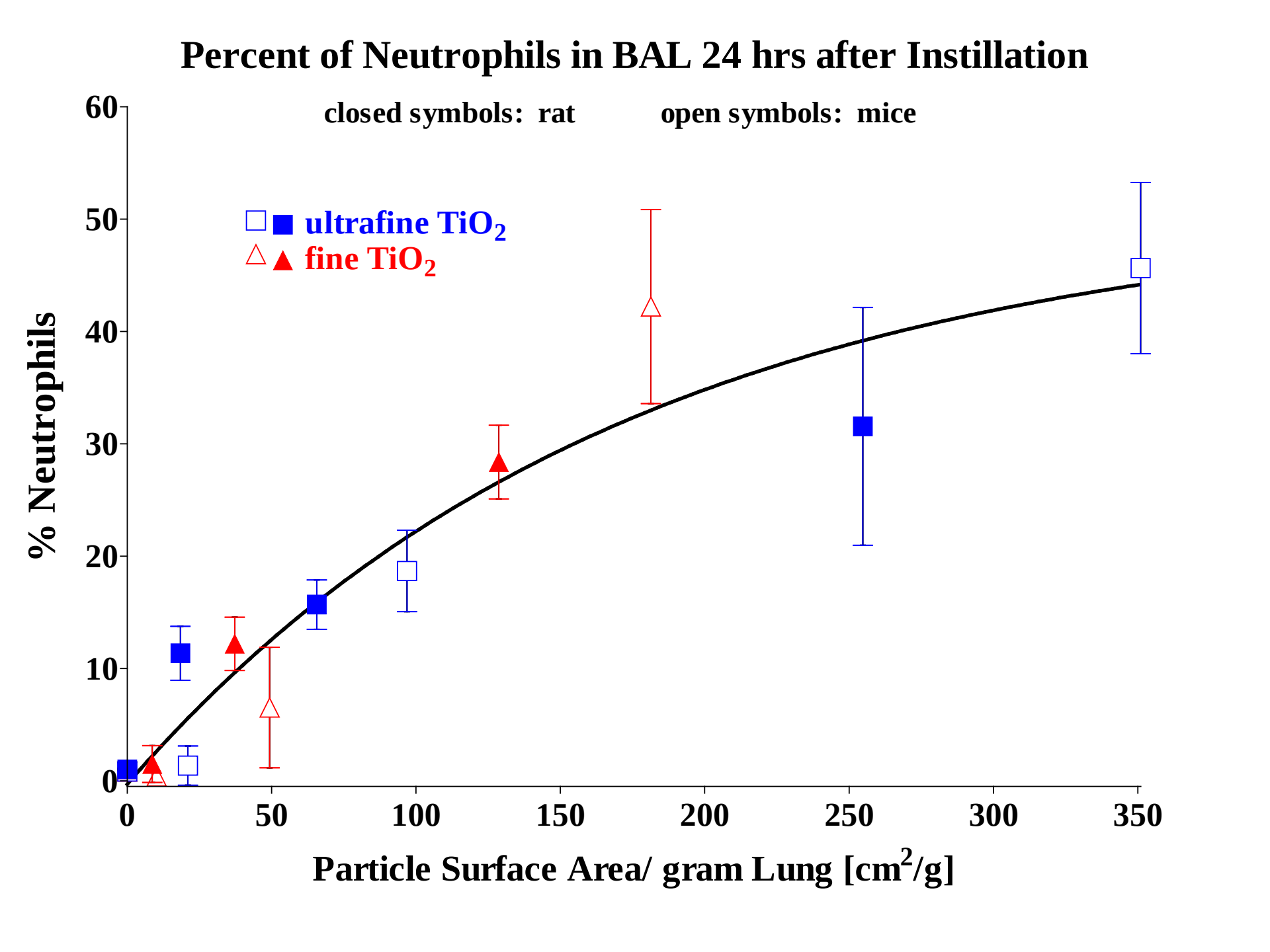
Particle Mass ***vs*** ***Particle Number***

Which Dose-Metric?

Percent of Neutrophils in BAL 24 hrs after Instillation of TiO_2 in Rats

Correlation with Particle Surface Area




Percent of Neutrophils in BAL 24 hrs after Instillation
closed symbols: rat
open symbols: mice
ultrafine TiO2
fine TiO2
% Neutrophils
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350
Particle Surface Area/ gram Lung [cm2/g]

In addition: "Chemical" Dose-Metric,

***e.g.*, ROS inducing potential:**

DCFH-DA *(2'-7' dichlorofluorescin-diacetate)* **assay**

FRAS *(ferric reducing ability of serum)* **assay**

Vit C assay

others…

(as screening tool for categorization of NPs based on reactivity [Bello et al., 2009; Rushton et al., 2010])

ROS-Inducing Capacity of NanoTiO_2 depends on Crystalline State:

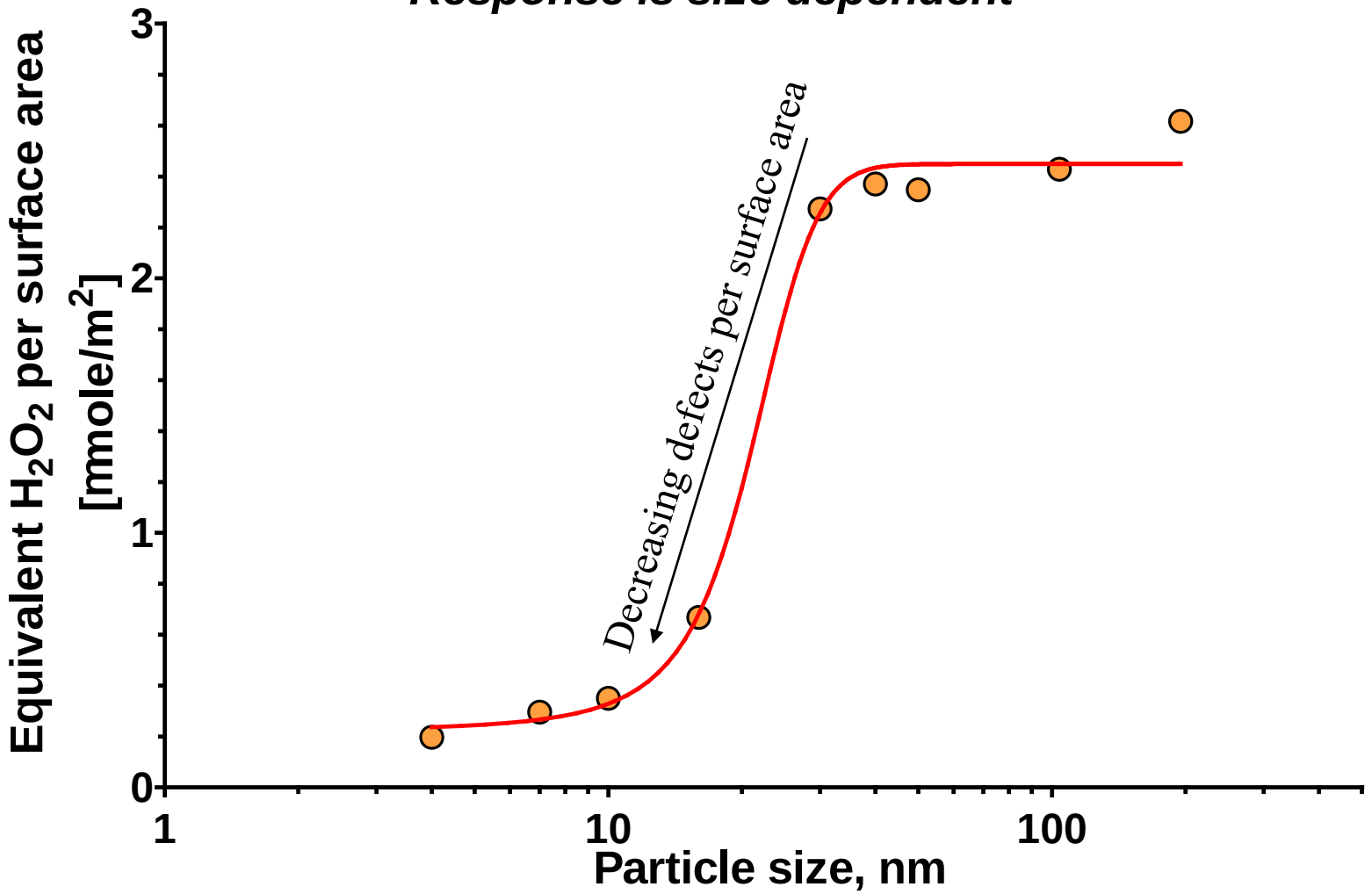
amorphous >

anatase >

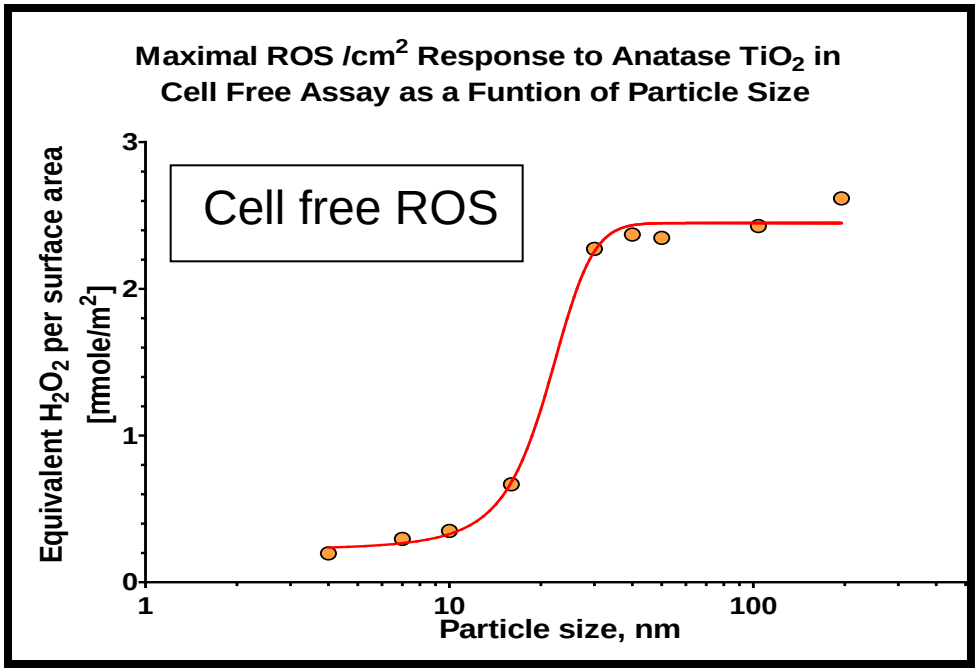
anatase/rutile mixture >

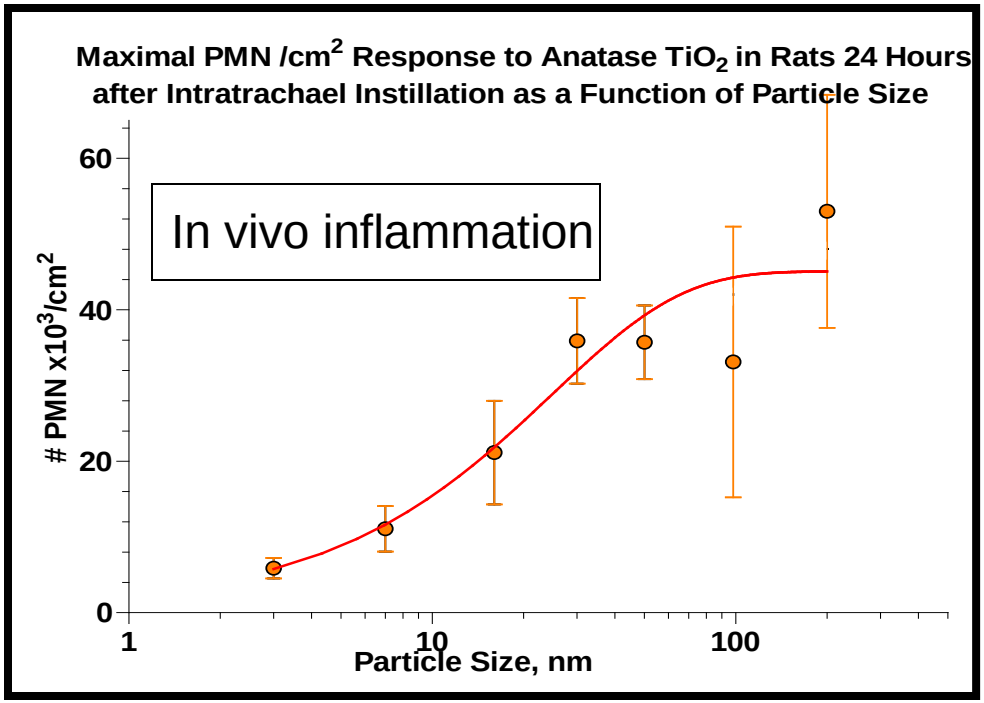
rutile

ROS /cm^2 Response of Anatase TiO_2 in Cell Free Assay as a Funtion of Particle Size: *Response is size dependent*

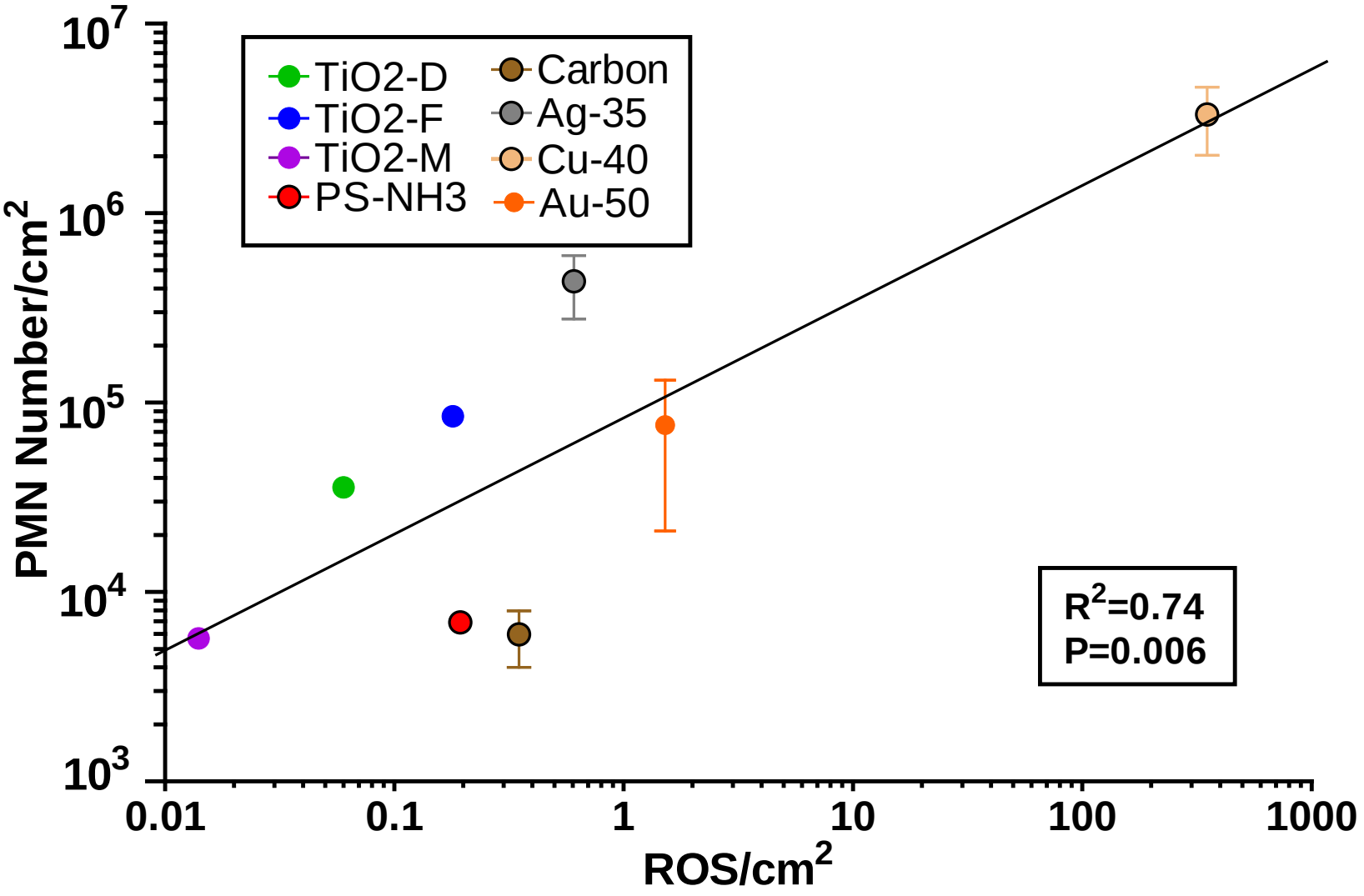


Jiang et al, 2008


Maximal ROS /cm2 Response to Anatase TiO2 in
Cell Free Assay as a Funtion of Particle Size
Cell free ROS
Equivalent H2O2 per surface area
[mmole/m2]
0
1
2
3
1
10
100
Particle size, nm


Maximal PMN /cm2 Response to Anatase TiO2 in Rats 24 Hours
after Intratrachael Instillation as a Function of Particle Size
In vivo inflammation
PMN x10^3/cm^2
0
20
40
60
1
10
100
Particle Size, nm

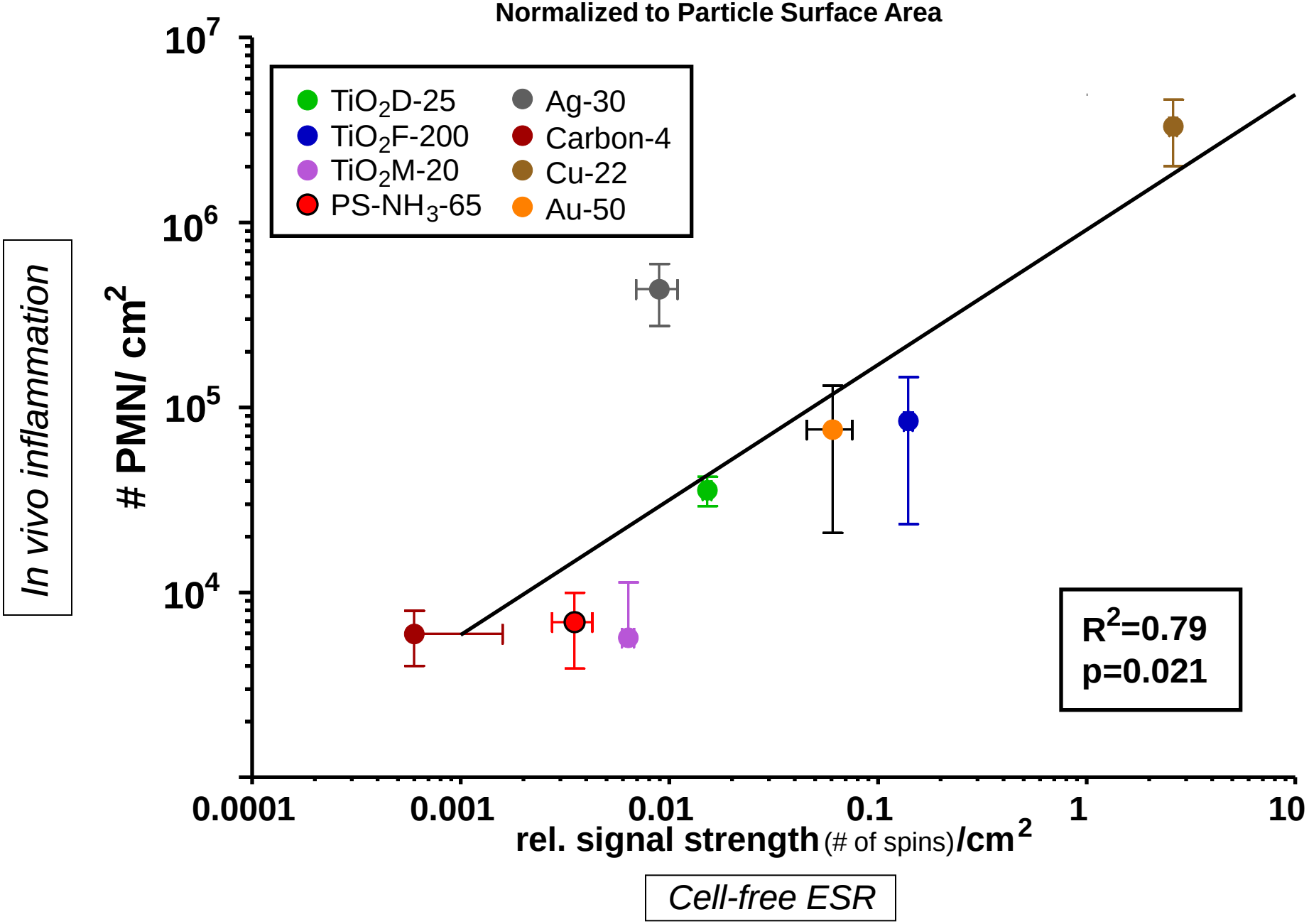
Cell-free ROS *(DCFH oxidation)* **Response vs In Vivo Rat PMN** *(Intratracheal Instill)*
Response to Nanoparticles
Normalized to Particle Surface Area



In vivo inflammation

In vitro Cell- free ROS

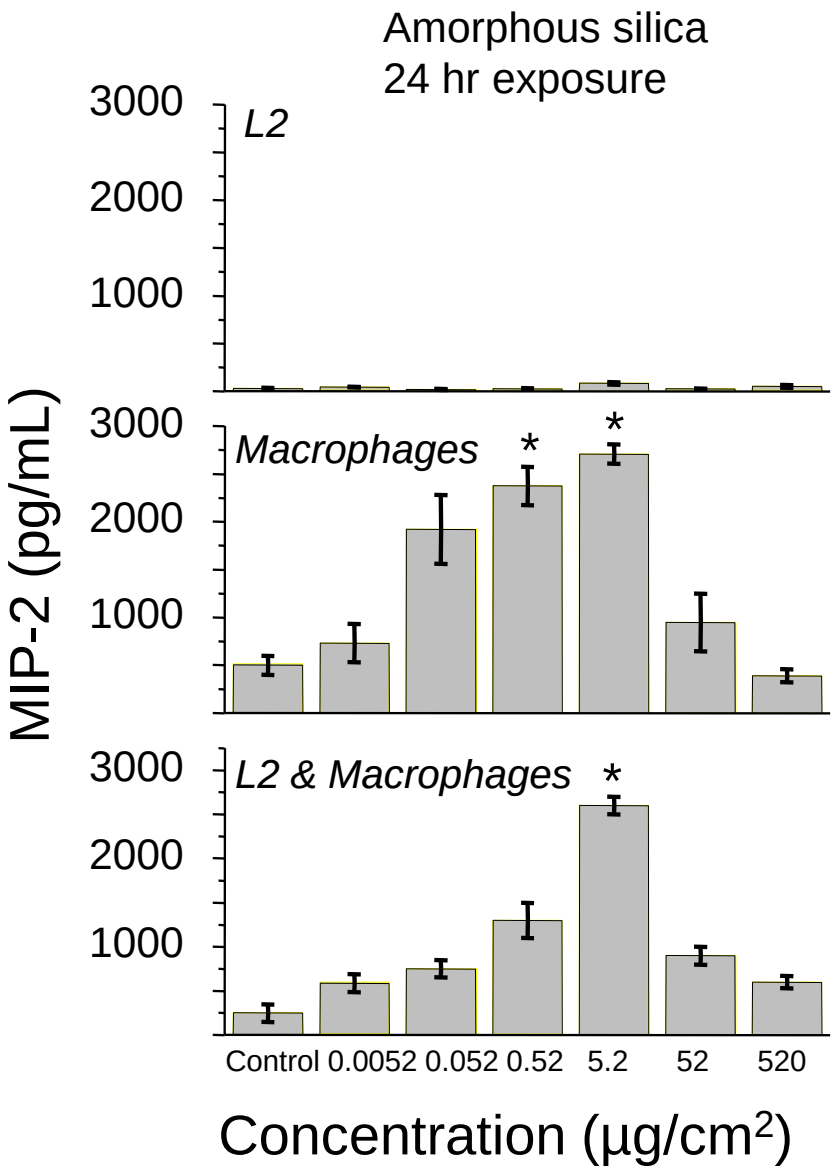
Rushton et al.2010

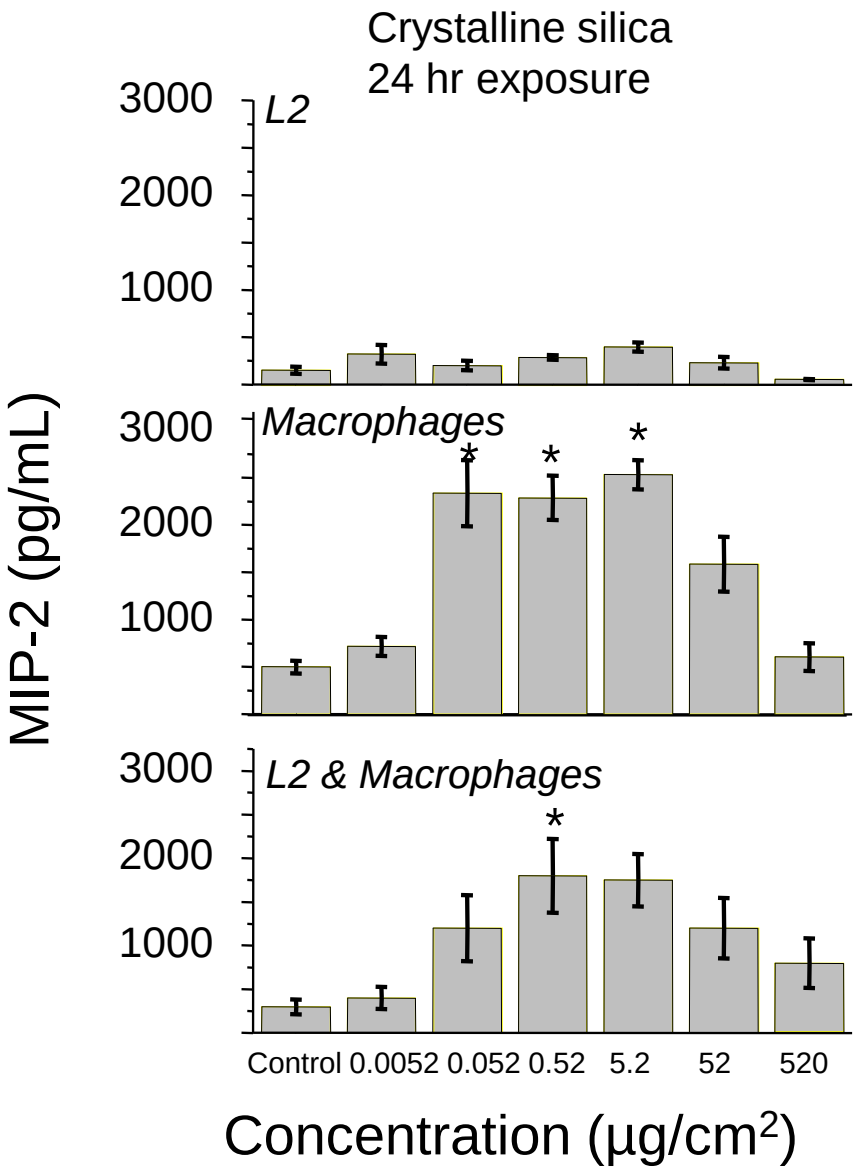

Cell-free ESR (DMPO Spin Trapping) Response vs In Vivo Rat PMN (Intratrach. Instill.)
Response to Nanoparticles
Normalized to Particle Surface Area
TiO2D-25
TiO2F-200
TiO2M-20
PS-NH3-65
Ag-30
Carbon-4
Cu-22
Au-50
R2=0.79
p=0.021
In vivo inflammation
PMN/ cm2
10^7
10^6
10^5
10^4
0.0001
0.001
0.01
0.1
1
10
rel. signal strength (# of spins)/cm2
Cell-free ESR

ASSESSING TOXICITY OF FINE AND NANOPARTICLES

Crystalline Silica; Amorphous Silica; Nano Zinc Oxide; Fine Zinc Oxide

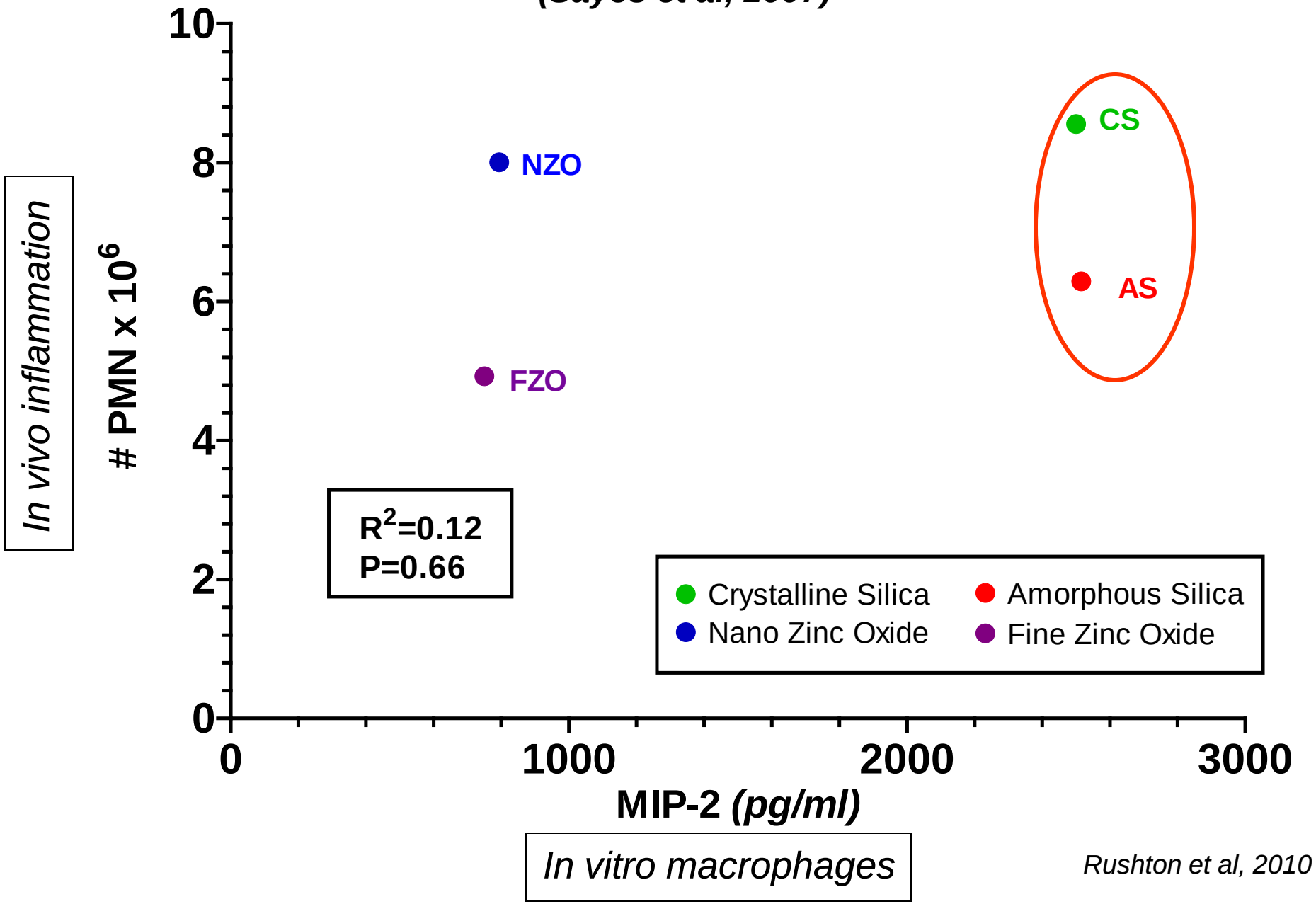
in vitro system, lung epithelial cells (*Sayes et al., 2007)*





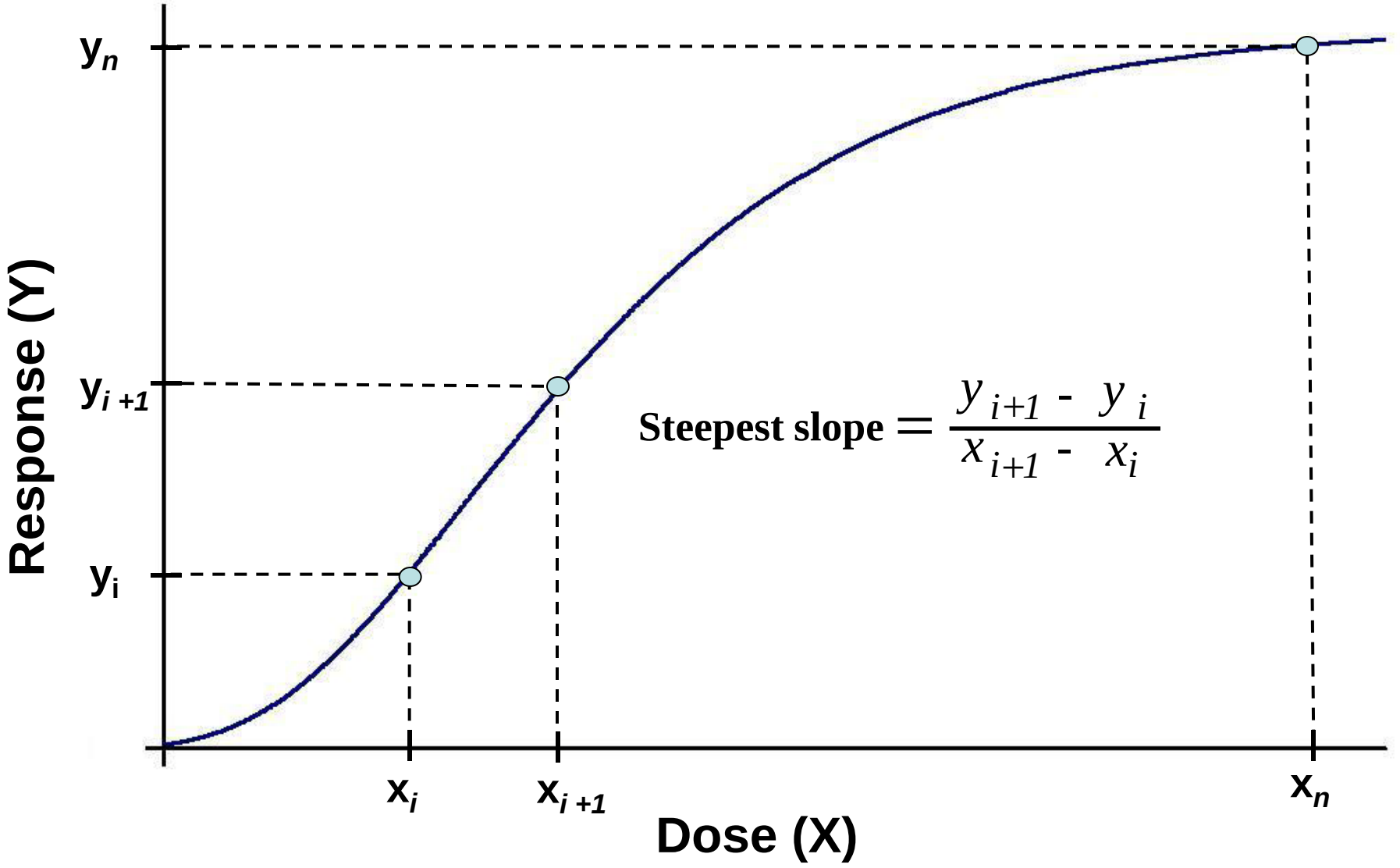
In Vivo/In Vitro Correlation, Highest Measured Responses

(Sayes et al, 2007)

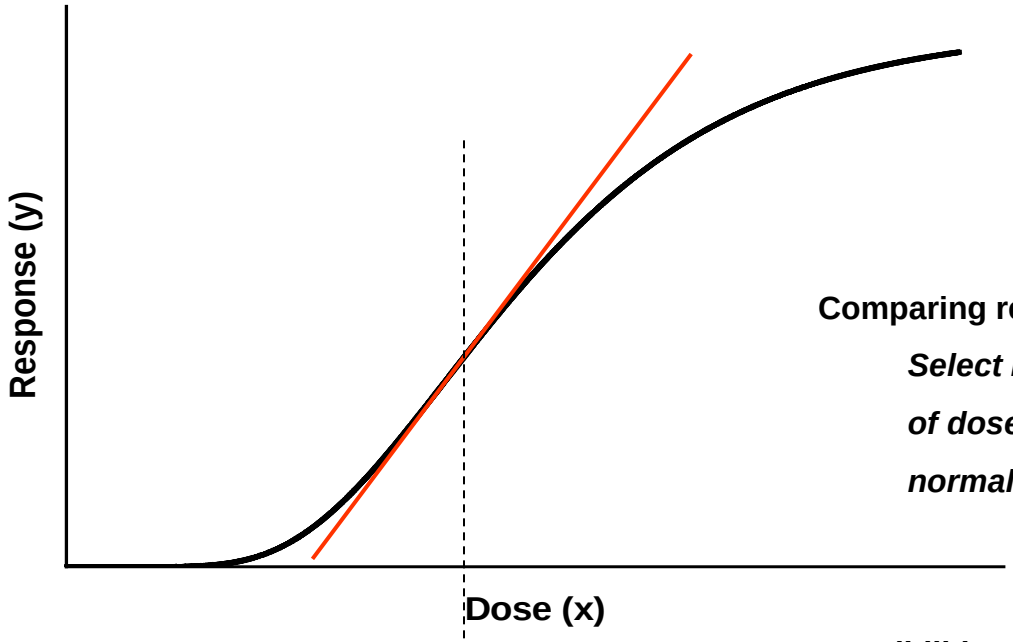


Rushton et al, 2010

Slope (response per unit dose) is Dose Dependent



Rushton et al, 2010

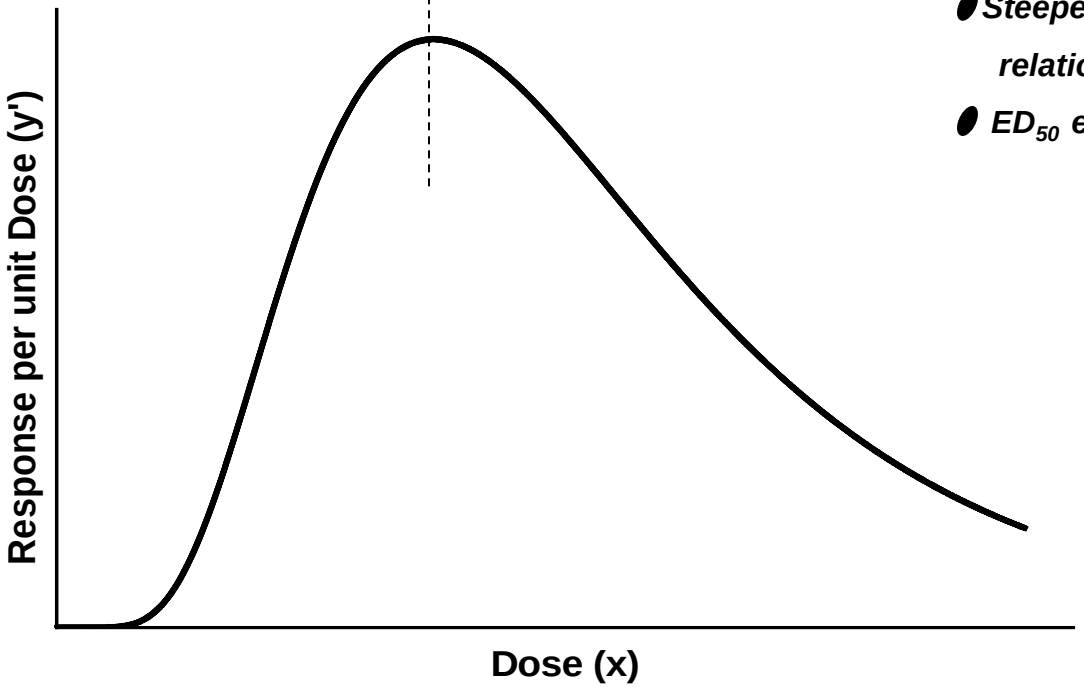


Comparing responses from different assays:

Select response at corresponding points of dose response curves of assays, normalized to a unit of dose.

Possibilities:

- ***Steepest slope of linear dose response relationship (Max of derivative)***
- ***ED_{50} equivalent of log dose response***



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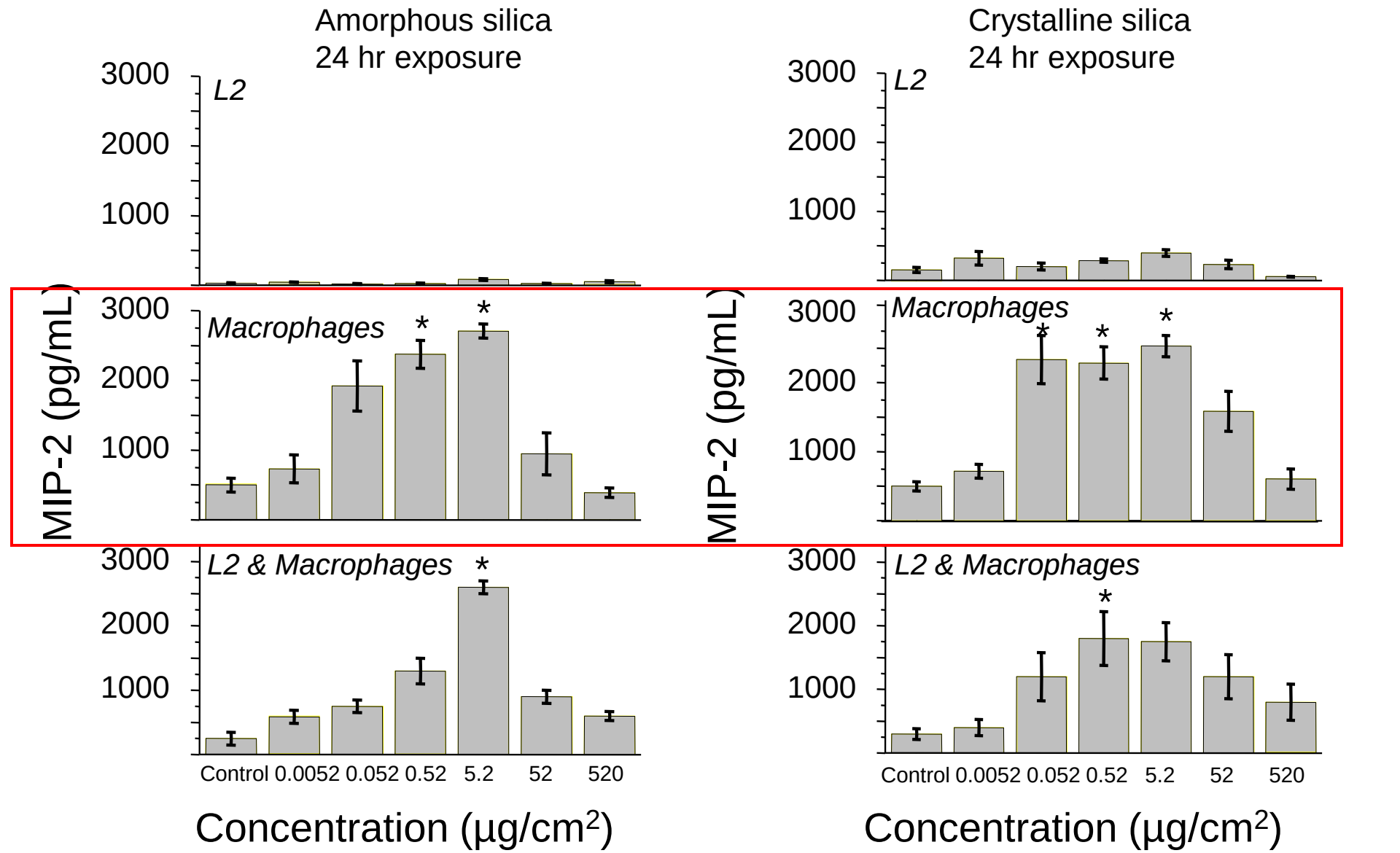
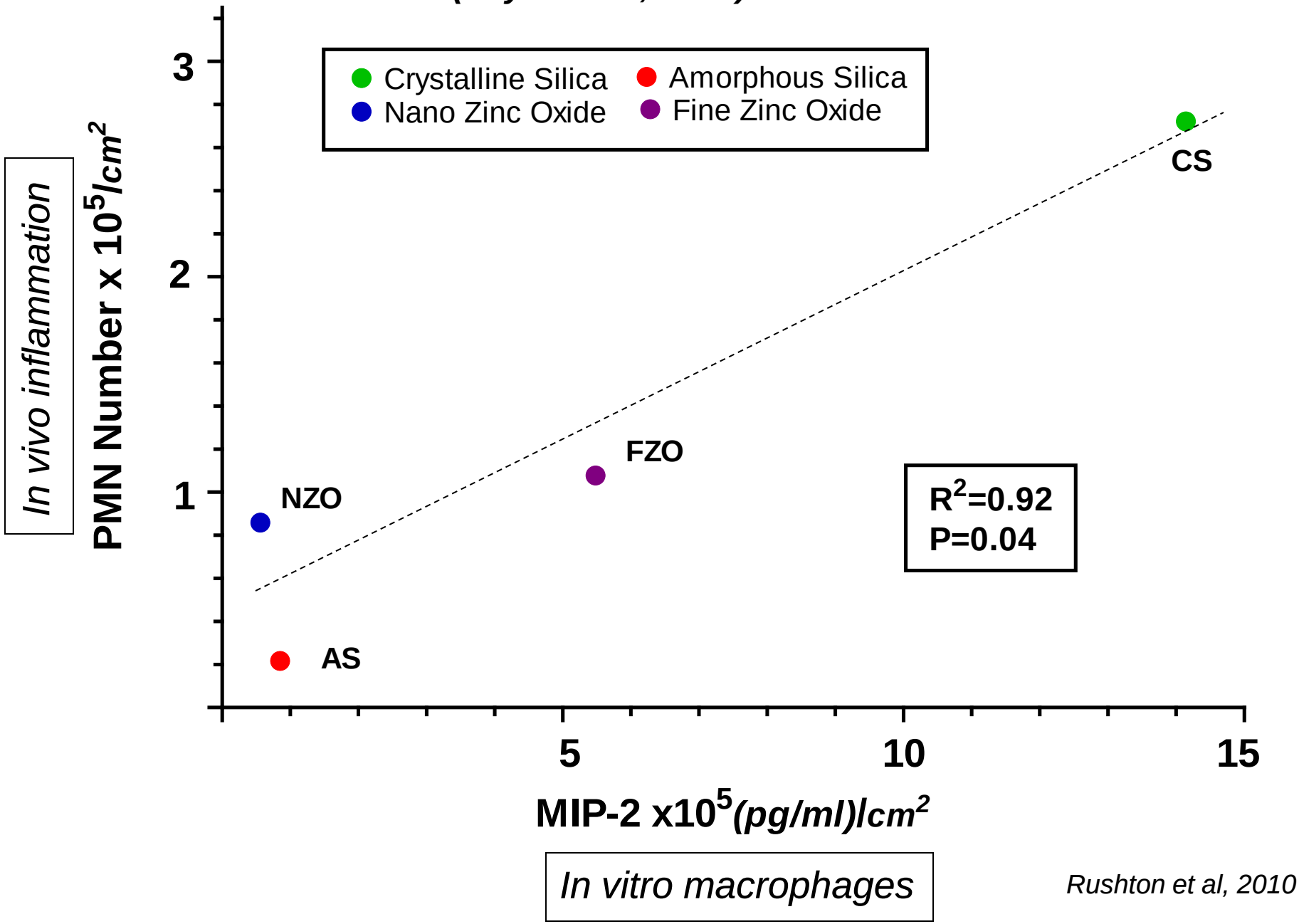
in vitro system, lung epithelial cells (*Sayes et al., 2007)*

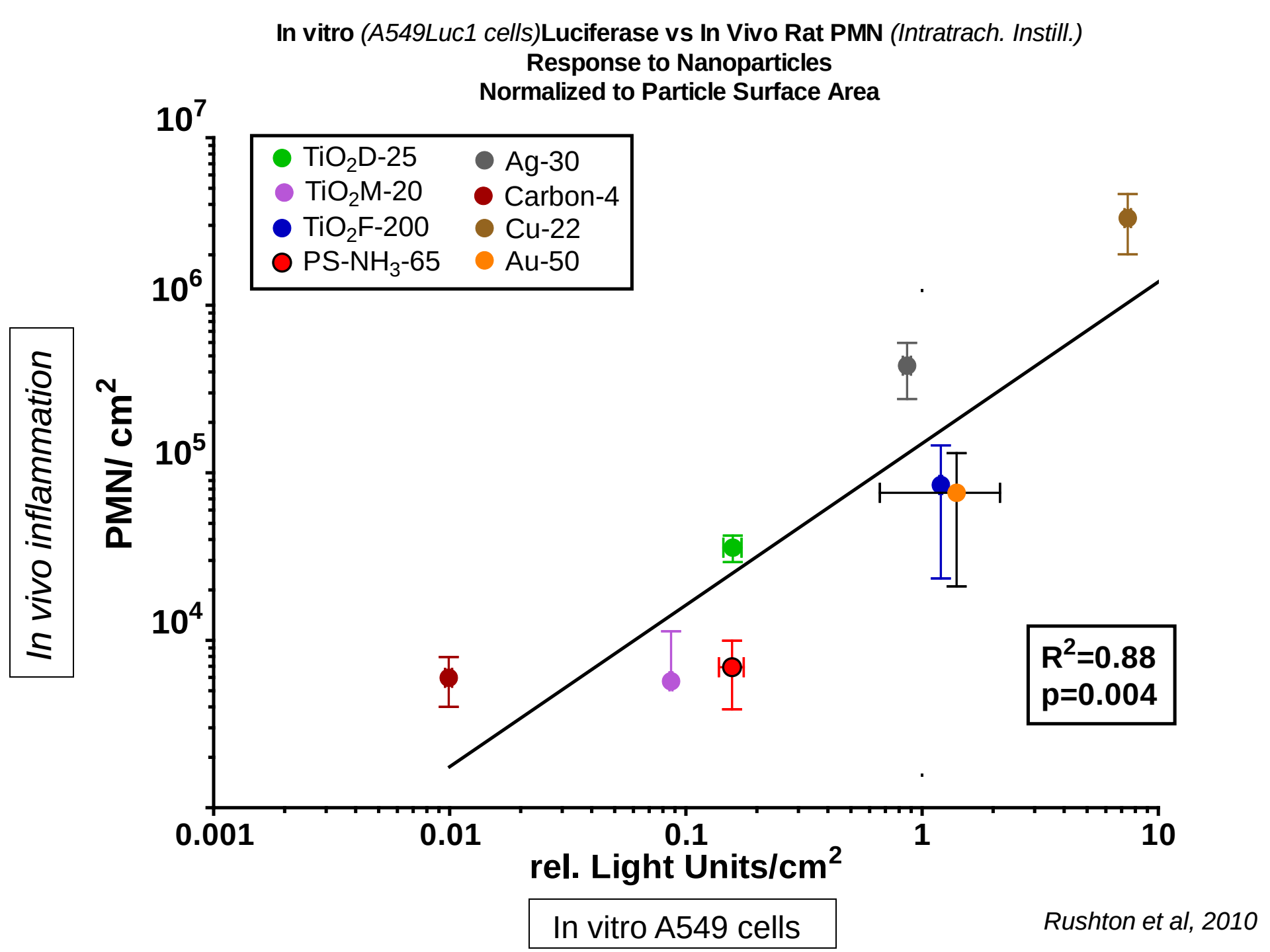


In Vivo/In Vitro Correlation, Highest Responses Per NP Surface Area

(Sayes et al, 2007)



Rushton et al, 2010


In vitro (A549Luc1 cells)Luciferase vs In Vivo Rat PMN (Intratrach. Instill.)
Response to Nanoparticles
Normalized to Particle Surface Area
TiO2D-25
TiO2M-20
TiO2F-200
PS-NH3-65
Ag-30
Carbon-4
Cu-22
Au-50
In vivo inflammation
PMN/ cm2
10^7
10^6
10^5
10^4
0.001
0.01
0.1
1
10
rel. Light Units/cm2
R2=0.88
p=0.004
In vitro A549 cells
Rushton et al, 2010

Example of categorizing NPs by a hazard scale or by Reference Particle Equivalent

(based on maximum effect per NP surface, mass or number, as derived from dose-response curves)

NP-TYPE	SIZE	HAZARD CATEGORY
Carbon black	41 nm *(aggregated)*	Very low
TiO_2 (anat.)	20 nm *(aggregated)*	
Polystyrene	60 nm (positive charge)	
TiO_2 (anat./rut.)	25 nm *(aggreg/agglom)*	Low
Au	50 nm	
Ag	35 nm *(aggreg/agglom)*	High
Cu	40 nm *(aggreg/agglom)*	Very high

Example is based on pulmonary inflammatory response in rats (elicited PMN/cm^2).
Other *in vitro* or *in vivo* endpoints can be selected, *e.g.*, ROS/cm^2; LDH/cm^2; MN/cm^2; Prot.Aggr./cm^2 …

Example of categorizing NPs by a hazard scale or by Reference Particle Equivalent

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NP-TYPE	SIZE	HAZARD CATEGORY	HAZARD RANKING per cm^2 (Carbon Black = 1)
Carbon black	41 nm *(aggregated)*	Very low	**1**
TiO_2 (anat.)	20 nm *(aggregated)*		**5**
Polystyrene	60 nm (positive charge)		**9**
TiO_2 (anat./rut.)	25 nm *(aggreg/agglom)*	Low	**11**
Au	50 nm		**21**
Ag	35 nm *(aggreg/agglom)*	High	**80**
Cu	40 nm *(aggreg/agglom)*	Very high	**620**

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NP-TYPE	SIZE	HAZARD CATEGORY	HAZARD RANKING		
			per cm^2 (Carbon Black = 1)	per gram	per 1.33×10^{15} particles
Carbon black	41 nm *(aggregated)*	Very low	1	6.1	19.3
TiO_2 (anat.)	20 nm *(aggregated)*		5	3.4	2.6
Polystyrene	60 nm (positive charge)		9	6.3	1
TiO_2 (anat./rut.)	25 nm *(aggreg/agglom)*	Low	11	5.0	4.2
Au	50 nm		21	1.0	2.1
Ag	35 nm *(aggreg/agglom)*	High	80	13.3	12.2
Cu	40 nm *(aggreg/agglom)*	Very high	620	151	508

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Comparing Pulmonary Response to TiO_2 NPs in Rats with same dose administered by Instillation or Inhalation:

- ***bolus type vs aerosol delivery***
- ***high vs. low dose rate***
- ***"prepared" vs. "pristine" NPs***
- ***more central vs. more peripheral lung deposition***

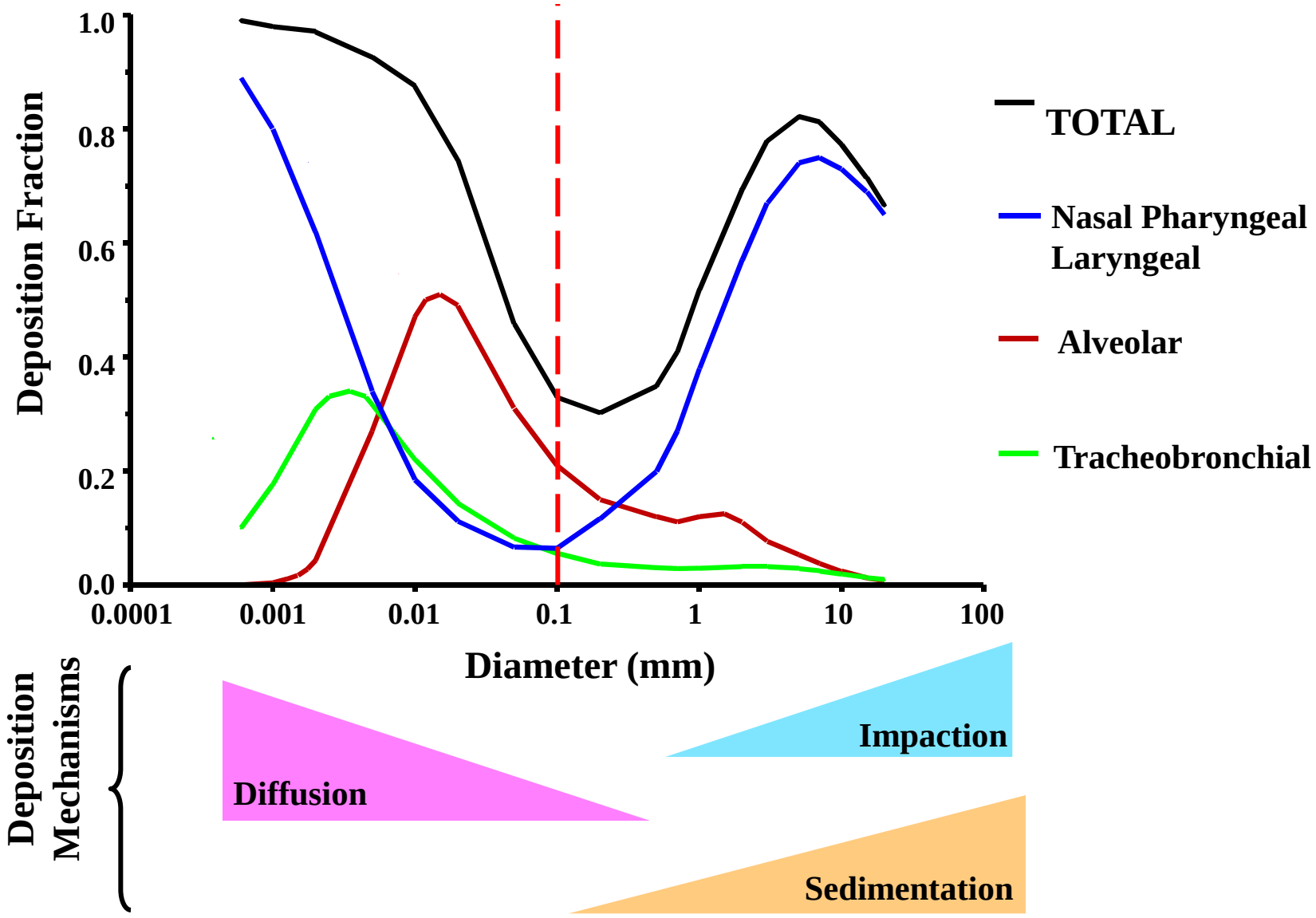
Dispersion as essential step when preparing NPs for toxicity testing using bolus type delivery in vitro and in vivo:

But:

Does it affect NP properties?

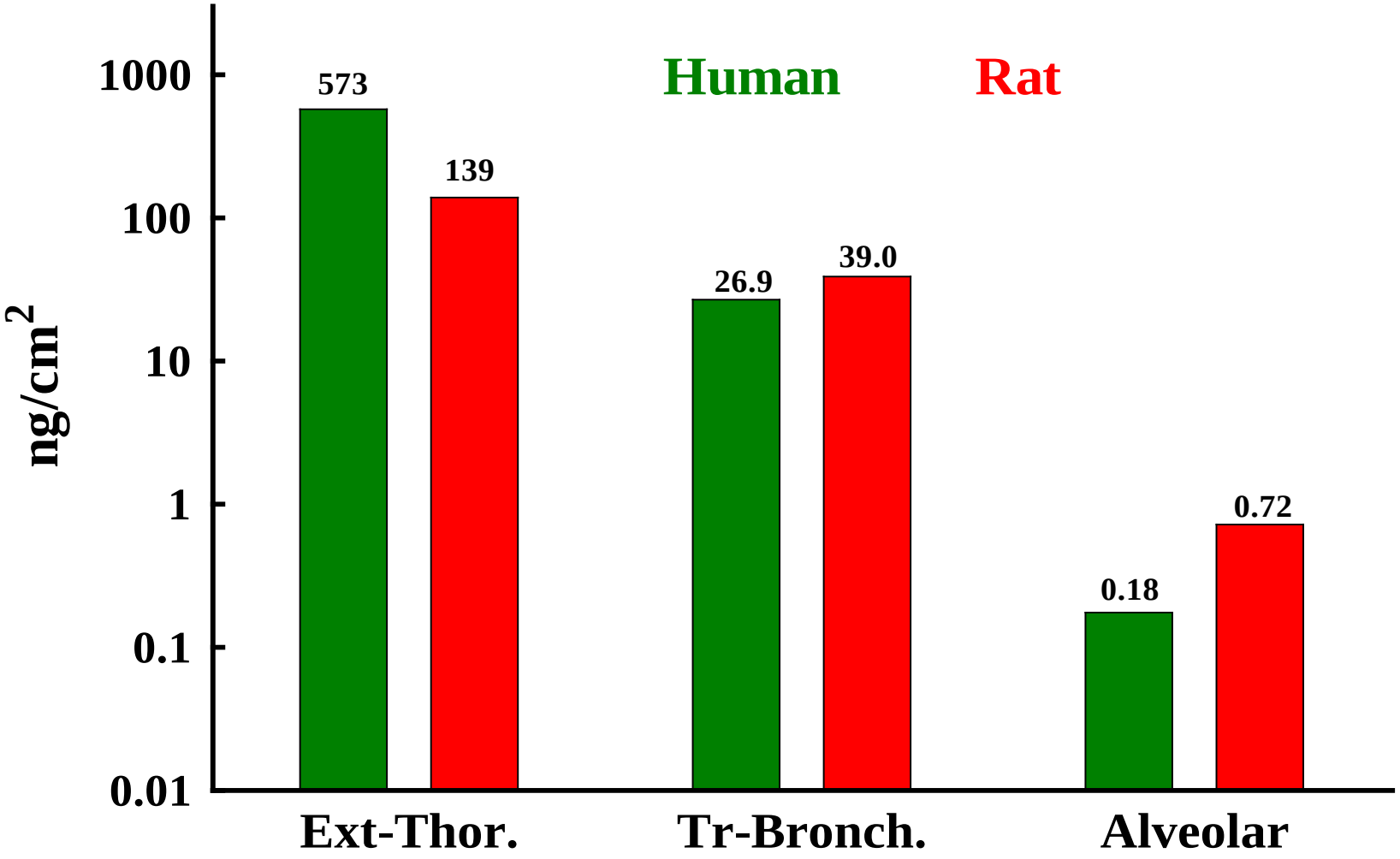
Which dispersant?

Which sonication system, time and power?

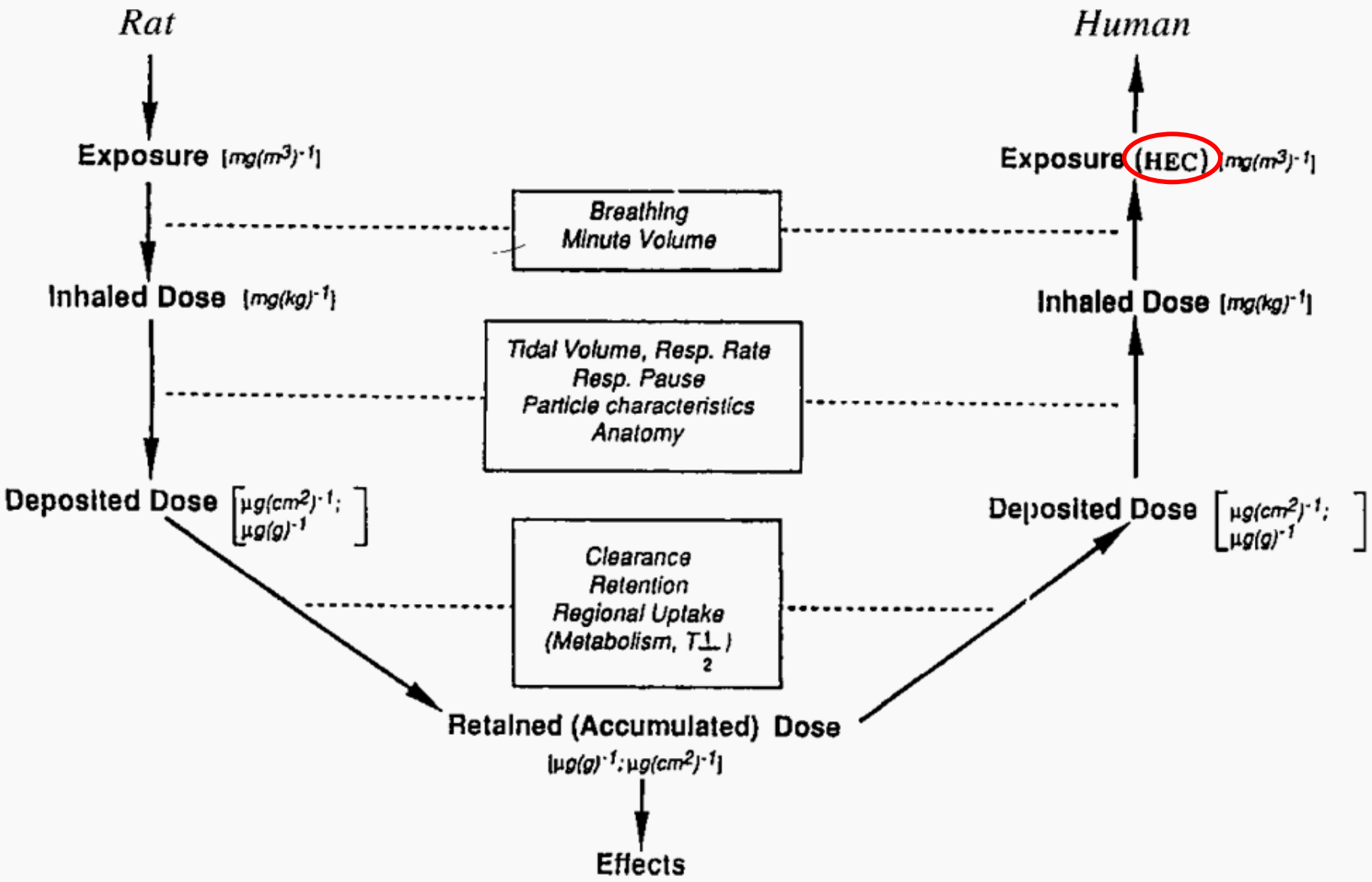

Deposition Fraction
1.0
0.8
0.6
0.4
0.2
0.0
0.0001
0.001
0.01
0.1
1
10
100
Diameter (mm)
TOTAL
Nasal Pharyngeal
Laryngeal
Alveolar
Tracheobronchial
Deposition
Mechanisms
Diffusion
Impaction
Sedimentation

Deposition per Unit Surface Area (cm^2) over 8 Hour Exposure at 100 mg/m^3 (nasal breathing, resting conditions)

Nanoparticle Size: CMD =20 nm; GSD = 1.0
Density: ρ = 1 g/cm^3



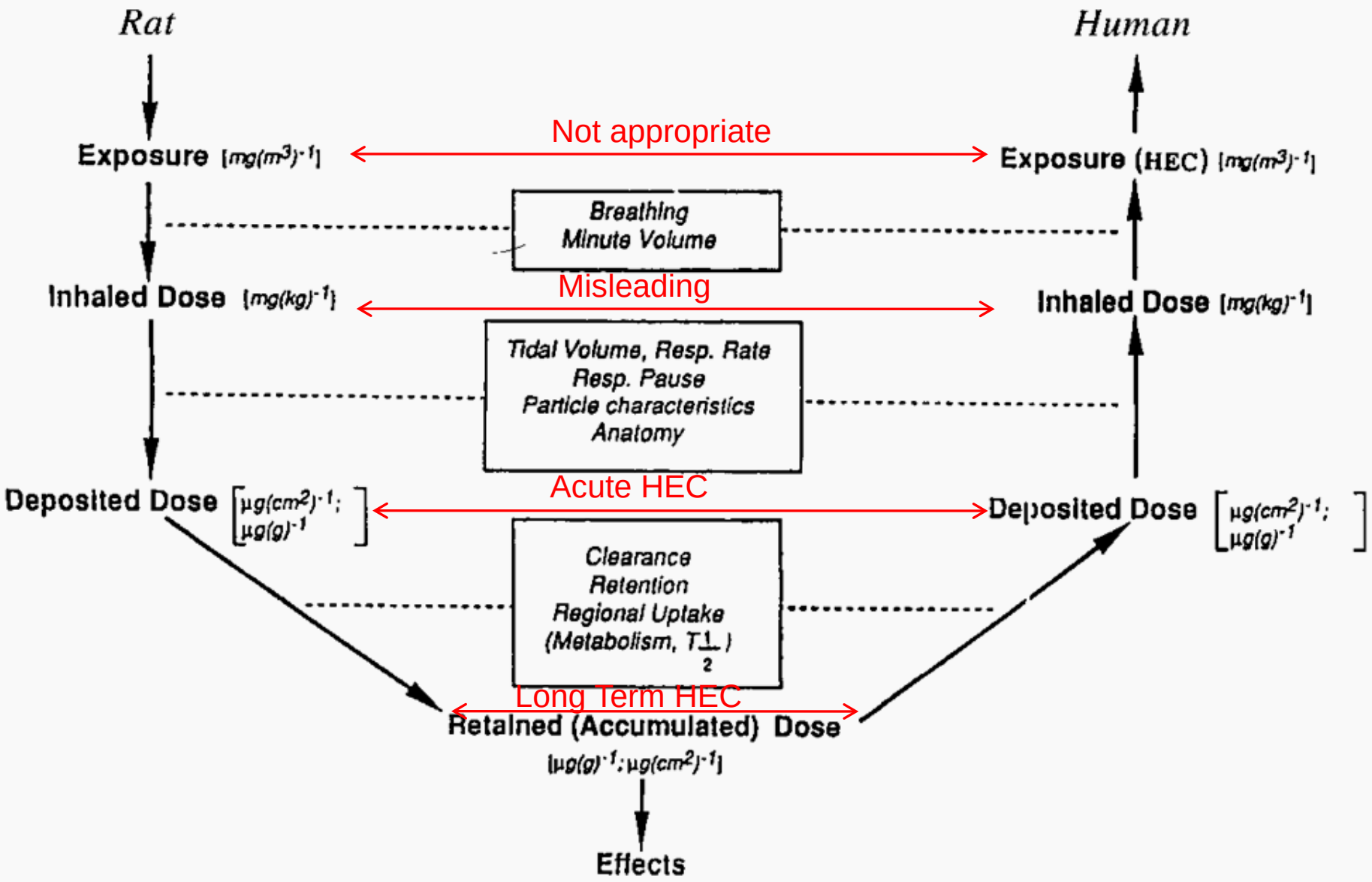
Dosimetric Extrapolation of Inhaled Particles from Rats to Humans



Assumption: If retained dose is the same in rats and humans, then effects will be the same

Oberdörster, 1989

Dosimetric Extrapolation of Inhaled Particles from Rats to Humans



Assumption: If retained dose is the same in rats and humans, then effects will be the same

Oberdörster, 1989

Risk = f (hazard; exposure)