ECHA
EUROPEAN CHEMICALS AGENCY

Topical Scientific Workshop on Regulatory Challenges in Risk Assessment of Nanomaterials

October 23-24, 2014
Helsinki, Finland

Topic 3: ***Metrology and dose metrics for hazard and exposure assessment throughout the life cycle***

Concepts of Nanoparticle Toxicology, Dosimetry and Risk Assessment

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UNIVERSITY of ROCHESTER MEDICAL CENTER

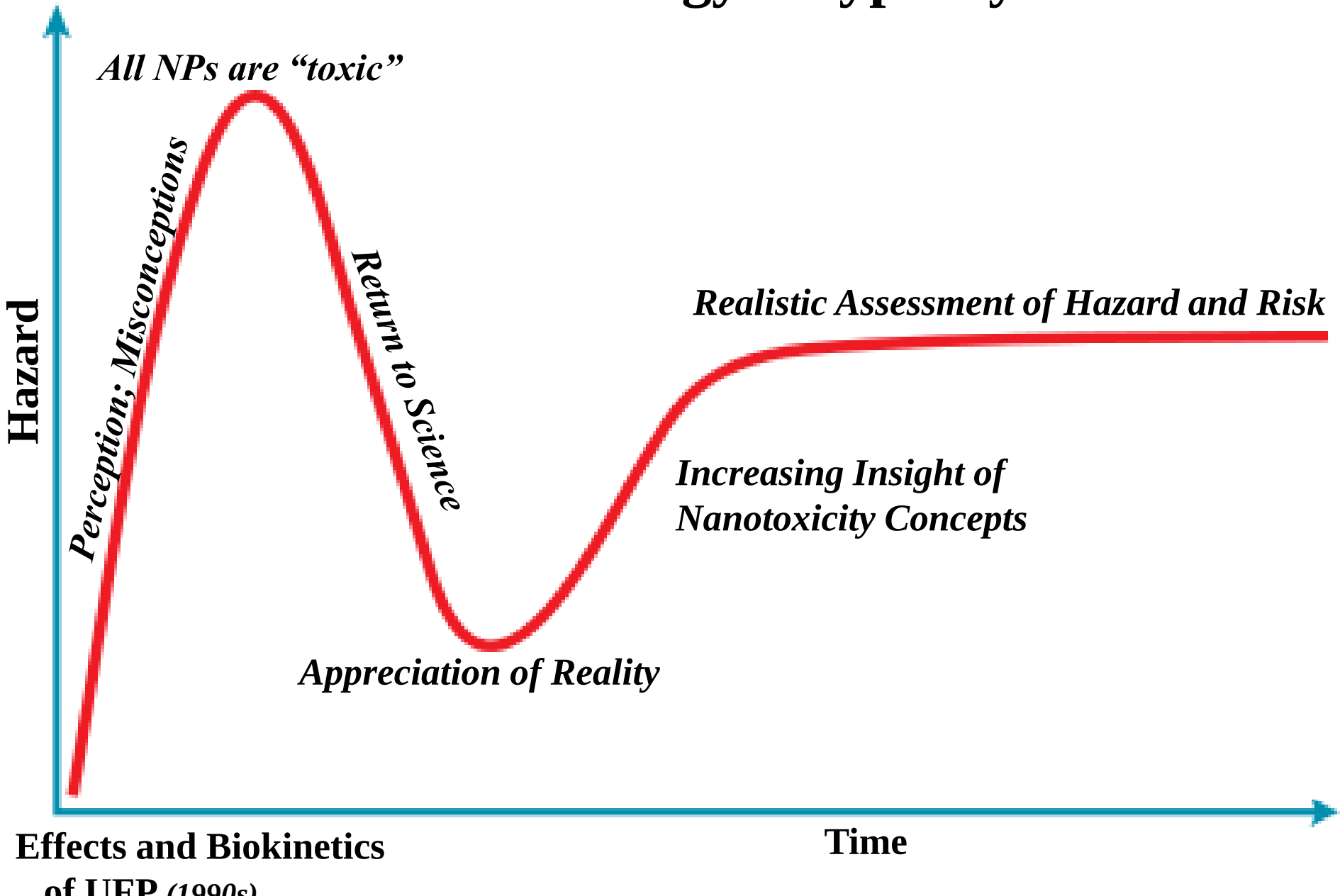
Concepts to be addressed:

— ***Key parameters of nanomaterials affecting hazard properties as basis for testing and for using metrics***

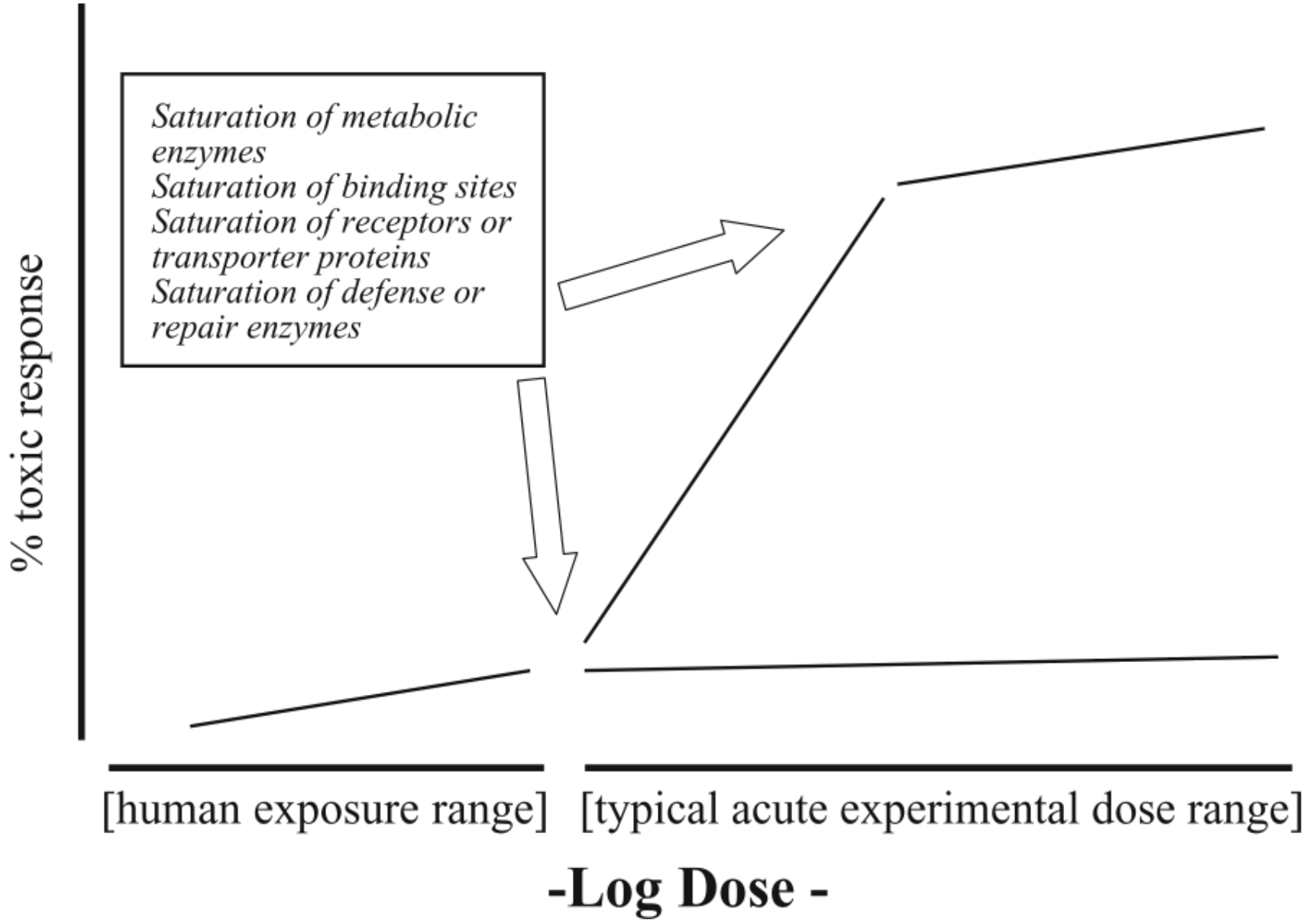
— ***Mode of action and choice of dosemetrics***

— ***Approach involving dosimetry and dosemetrics for regulatory hazard and risk characterization***

Nanotoxicology - Hype Cycle



Conceptual Depiction of Factors for Considering Dose-dependent Transitions in Determinants of Toxicity



From: Slikker Jr., et al. 2004

Toxicology 254 (2008) 82–90

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles

Jiangxue Wang[a,b], Ying Liu[a,b], Fang Jiao[a,b], Fang Lao[a,b], Wei Li[a,b], Yiqun Gu[c], Yufeng Li[a,b], Cuicui Ge[a,b], Guoqiang Zhou[a,b], Bai Li[a,b], Yuliang Zhao[a,b,*], Zhifang Chai[a,b], Chunying Chen[a,b,**]

[a] Laboratory for Bio-Environmental Effects of Nanomaterials and Nanosafety and Key Lab of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Aca
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Nanoparticles can be administered via nasal, oral, intraocular, intratracheal (pulmonary toxicity), tail vein and other routes. Here, we focus on the time-dependent translocation and potential damage of TiO_2 nanoparticles on central nervous system (CNS) through intranasal instillation. Size and structural properties are important to assess biological effects of TiO_2 nanoparticles. In present study, female mice were intranasally instilled with two types of well-characterized TiO_2 nanoparticles (i.e. 80 nm, rutile and 155 nm, anatase; purity > 99%) every other day. Pure water instilled mice were served as controls. The brain tissues were collected and evaluated for accumulation and distribution of TiO_2, histopathology, oxidative stress, and inflammatory markers at post-instillation time points of 2, 10, 20 and 30 days. The titanium contents in the sub-brain regions including olfactory bulb, cerebral cortex, hippocampus, and cerebellum were determined by inductively coupled plasma mass spectrometry (ICP-MS). Results indicated that the instilled TiO_2 directly entered the brain through olfactory bulb in the whole exposure period, especially deposited in the hippocampus region. After exposure for 30 days, the pathological changes were observed in the hippocampus and olfactory bulb using Nissl staining and transmission electron microscope. The oxidative damage expressed as lipid peroxidation increased significantly, in particular in the exposed group of anatase TiO_2 particles at 30 days postexposure. Exposure to anatase TiO_2 particles also produced ...

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Nano TiO_2 repeated bolus instillation into mouse:
7.5 mg into mouse = 17.5 grams into human nose!

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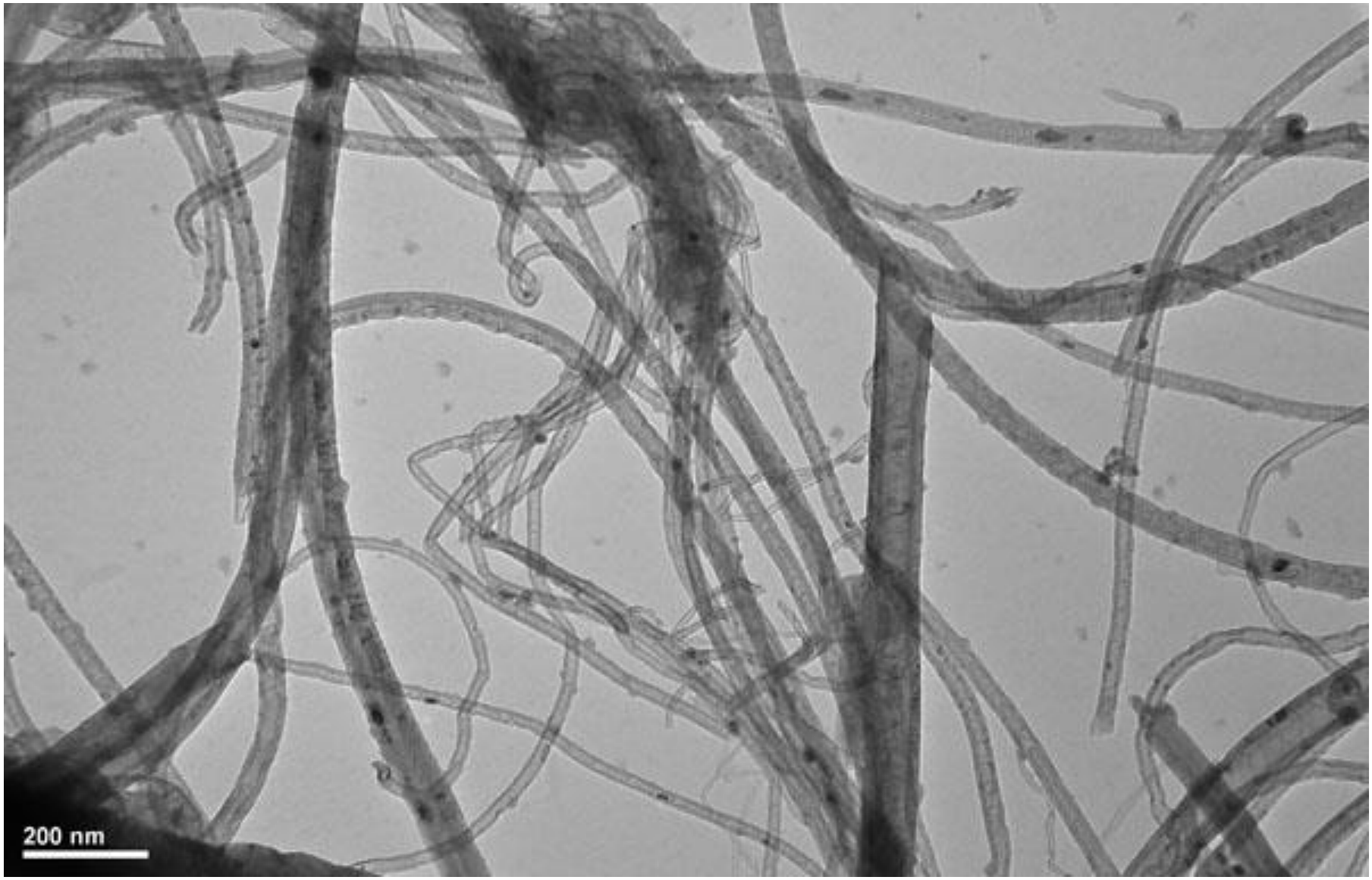
...


50 mL
YREX®
USA
mL 0
± 5%
200 mL
± 5%
50
150
100
100
150
50
17.5 g TiO_2 (P25)

DPPC/Alb-Dispersed MITSUI Multiwalled Carbon Nanotubes (MWCNTs)



Bolus Dosing of MWCNT: Granulomat. Inflam. and Mesothelioma = Asbestos like?

Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube

Takagi et al., J. Toxicol. Sci. 33 (No. 1): 105-116, 2008

Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study

Poland et al., Nature Nanotechnology 3, 423-428, 2008

Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats

Sakamoto et al, J.Tox. Sci., 34, 65-76, 2009

Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice

Takagi et al, Cancer Sci., 103(8), 1440-1444, 2012

Length-dependent pleural inflammation and parietal pleural responses after deposition of carbon nanotubes in the pulmonary airspaces of mice

Murphy et al, Nanotoxicology 7(6), 2013

Physico-chemical NP Properties Affecting Hazard Potential

Size *(aerodynamic, hydrodynamic)*

Size distribution

Shape

Agglomeration/aggregation

Density (material, bulk)

Surface properties:

- area *(porosity)*
- charge
- reactivity
- chemistry *(coatings, contaminants)*
- defects

Solubility/Sol-Rate (*lipid, aqueous, in vivo*)

Crystallinity

Biol. contaminants (e.g. endotoxin)

Properties can change

-with: method of production
preparation process
storage (*aging*)

-when introduced into
physiol. media, organism

-throughout life-cycle
(from cradle to grave)

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physiol. media, organism

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(from cradle to grave)

Key parameter: Dose!

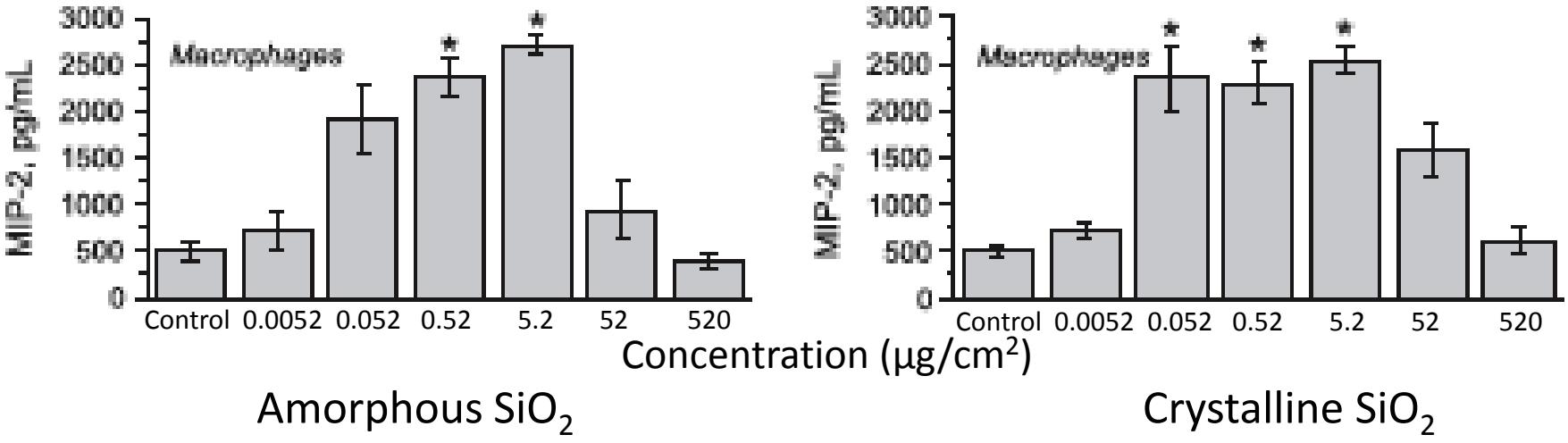
Expression of Dose?

Sayes et al., 2007:

ASSESSING TOXICITY OF FINE AND NANOPARTICLES *(In vitro and In vivo)*

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

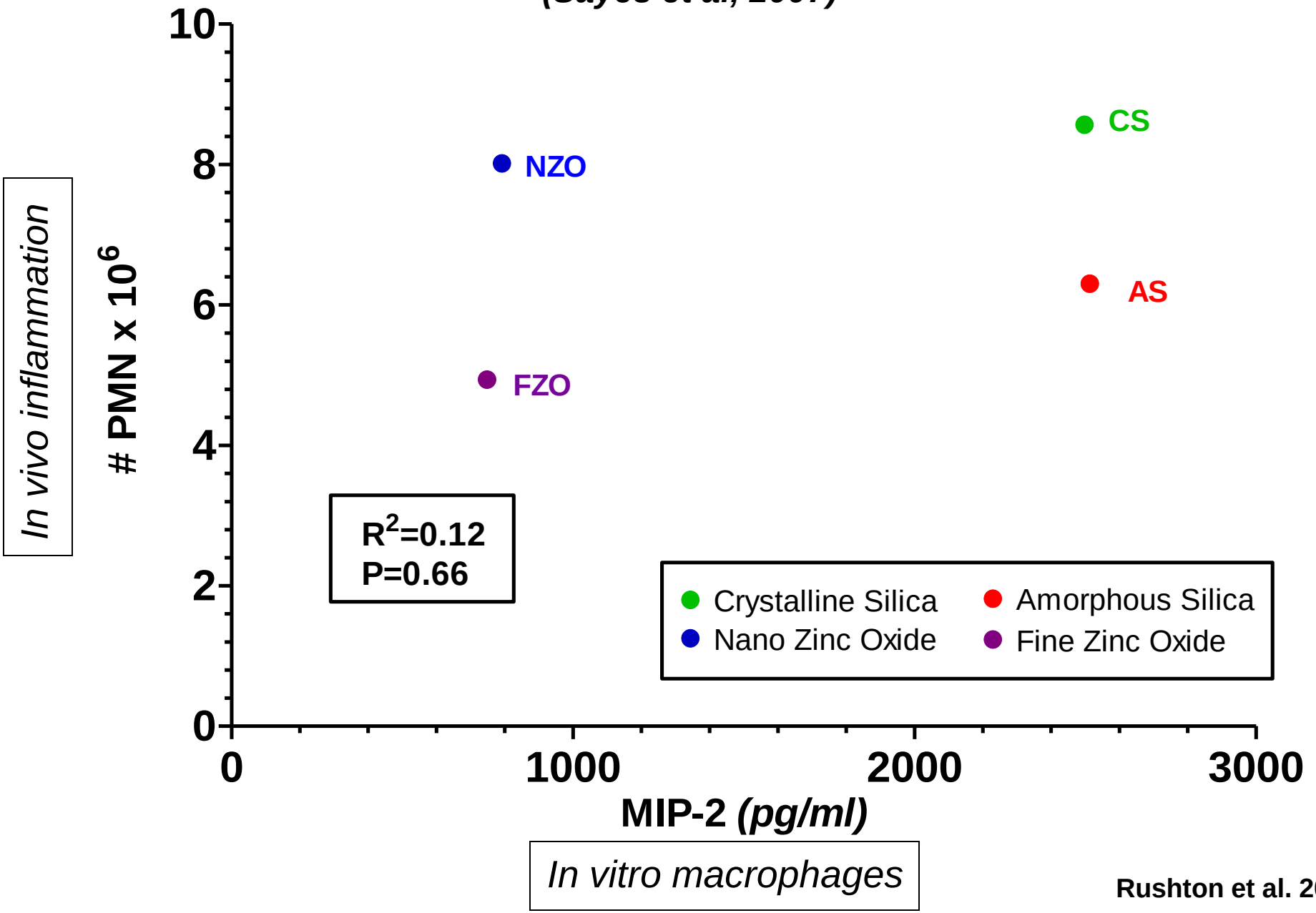
Rat Alveolar Macrophage Culture



Conclusion: ***comparison of in vivo and in vitro results demonstrated little correlation***

In Vivo/In Vitro Correlation, Highest Measured Responses

(Sayes et al, 2007)



Rushton et al. 2010

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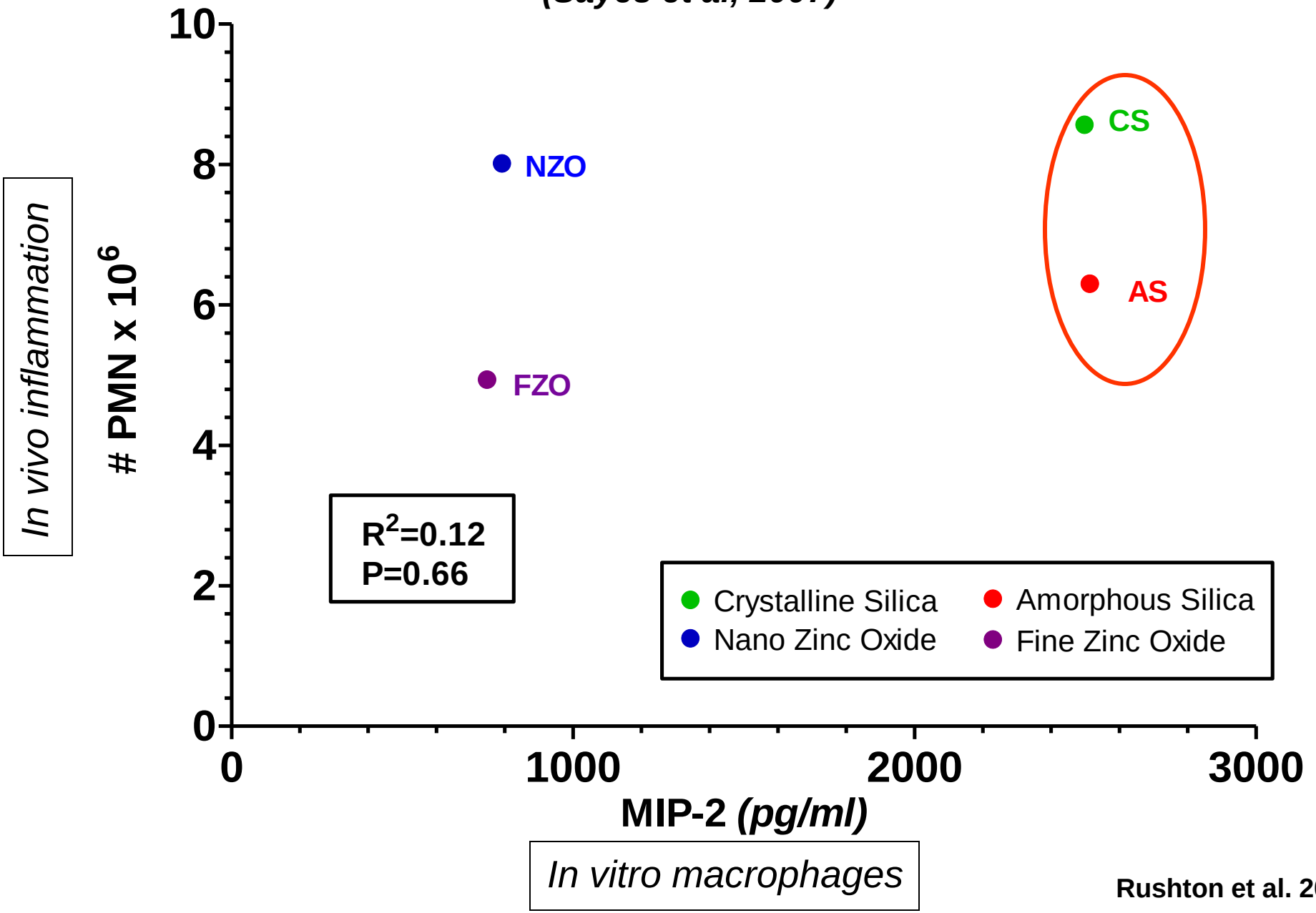
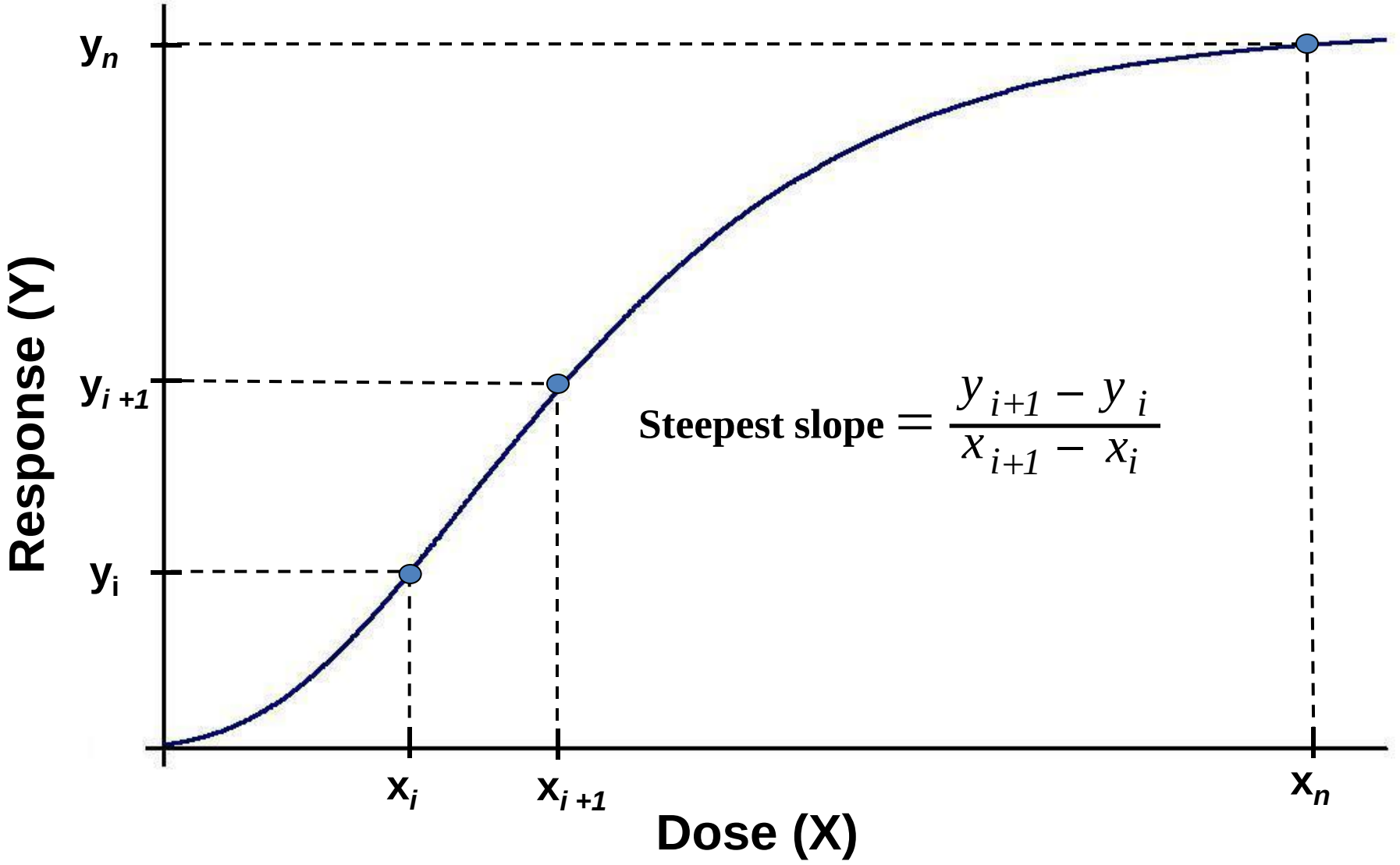


Rushton et al. 2010

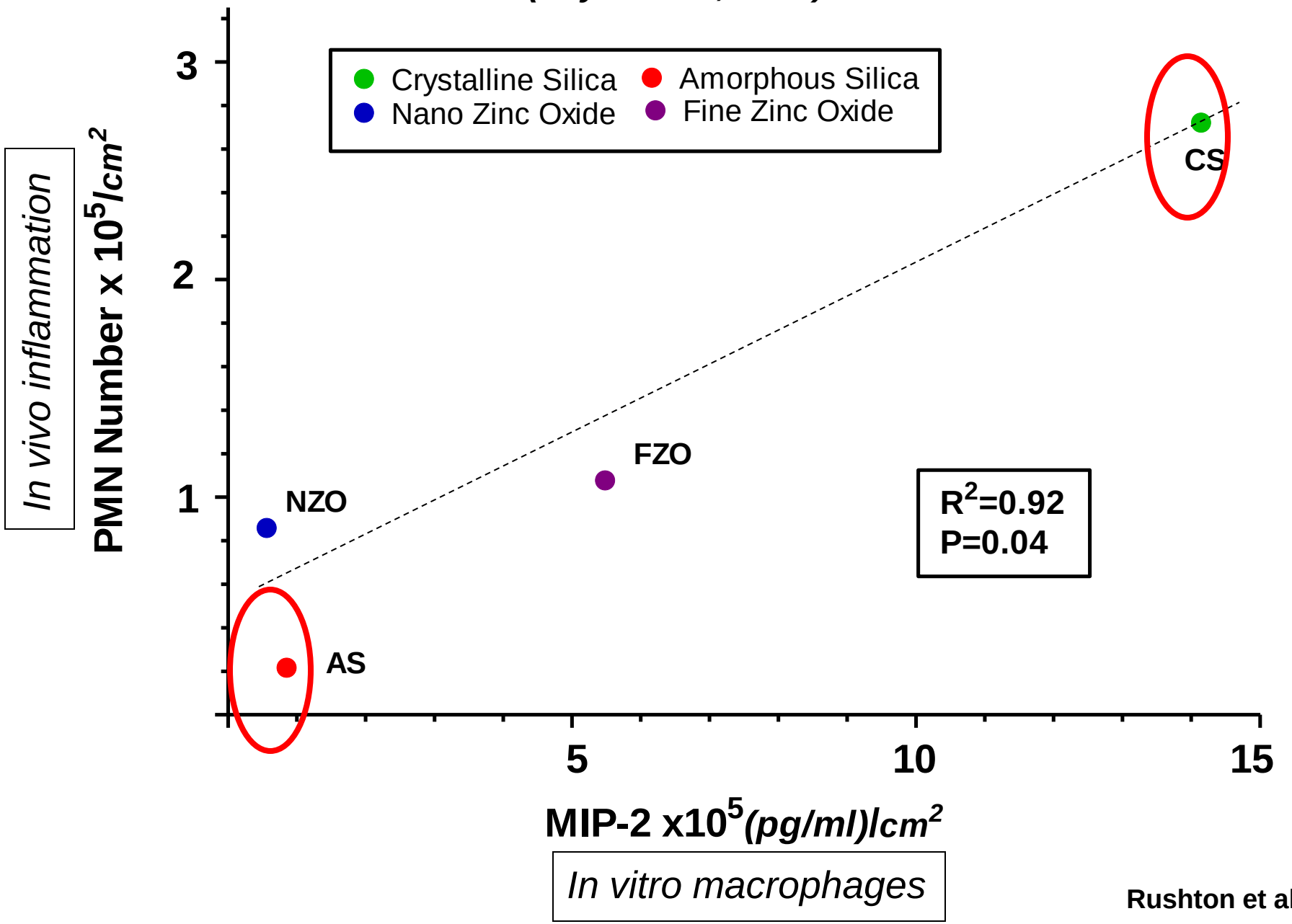
Dose-Response Analysis: Steepest slope *(max response/dose)* as indicator of toxicity



Rushton et al. 2010

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

(Sayes et al, 2007)



Rushton et al. 2010

Physical Dose-Metrics for NPs that Correlate with Biol./Toxicol. Effects *(Mode of Action)*:

Mass

Number

Surface Area
(as surrogate for surface props.)

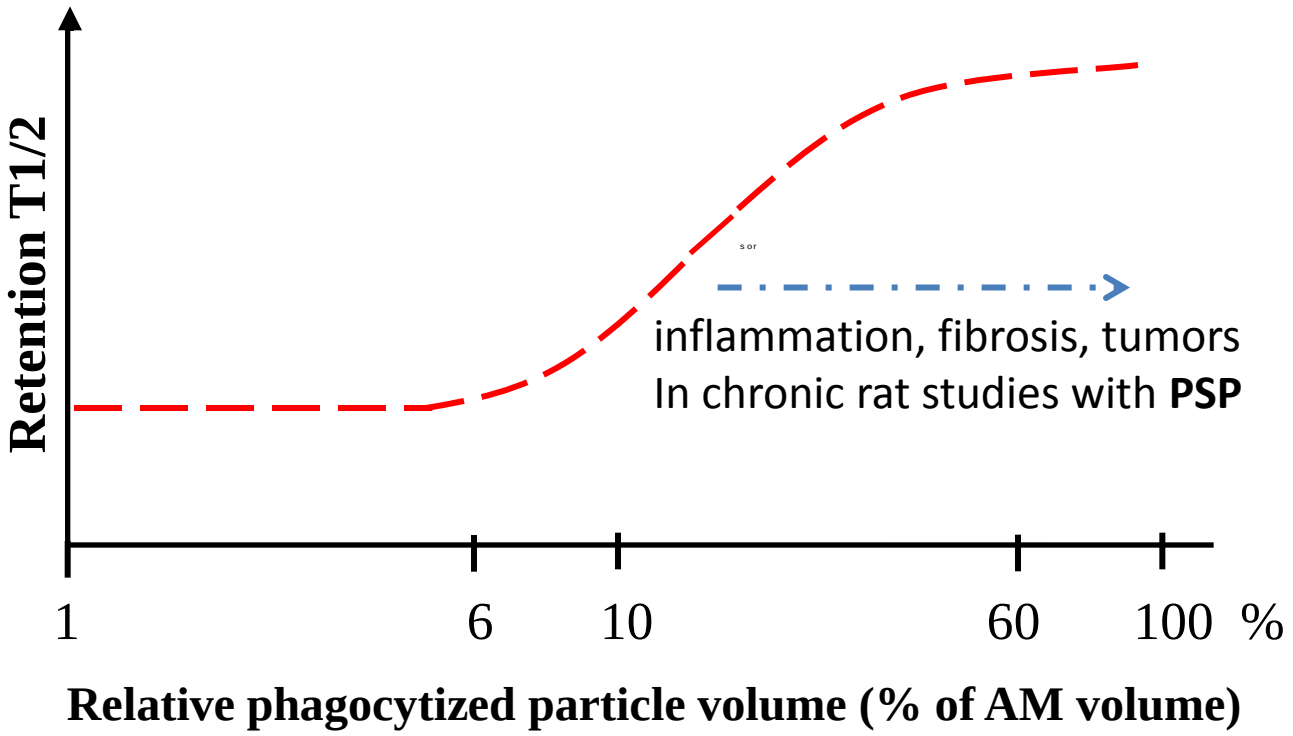
Volume

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

Particle Volume

Morrow Hypothesis (1988)**:**

Lung particle overload associated impairment of alveolar macrophage clearance function correlates with phagocytized particle volume.



Particle Volume

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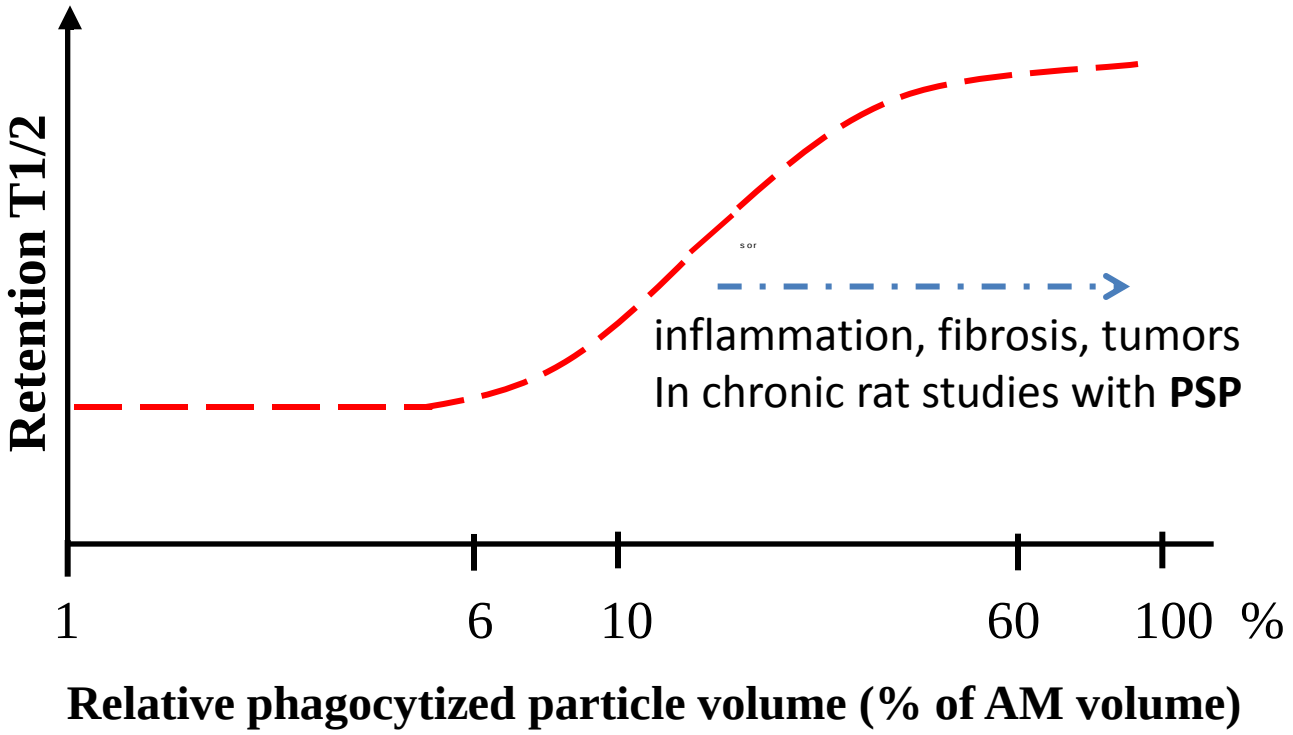
But: ***Only for Poorly Soluble Particles of low cytotoxicity (PSP)***



Lung Particle Overload, Nanoparticles and AM mediated Particle Clearance:

Does volumetric overload concept apply to nanoparticles?

12-Week Inhalation Exposure, Ultrafine and Fine TiO_2 and Cristobalite (SiO_2)

Retained dose/10^6 AM at end of exposure

	Mass	Volume		Surface	Number	Test Particle Retention
	µg	nl	% of AM volume	cm^2	x 10^{-9}	control = 1
Control	**0**	**0**	**0**	**0**	**0**	**1**
TiO_2 fine (250 nm)	**340**	**90**	**9**	**21.9**	**10.9**	**1.8***
TiO_2 ultrafine (25 nm)	**99.8**	**26**	**2.6**	**49.9**	**5420**	**8.2***
Cristobalite	**~20**	**7.6**	**0.76**	**2.4**		**28.8***

***Significantly different from control**

Oberdörster et al, 1994

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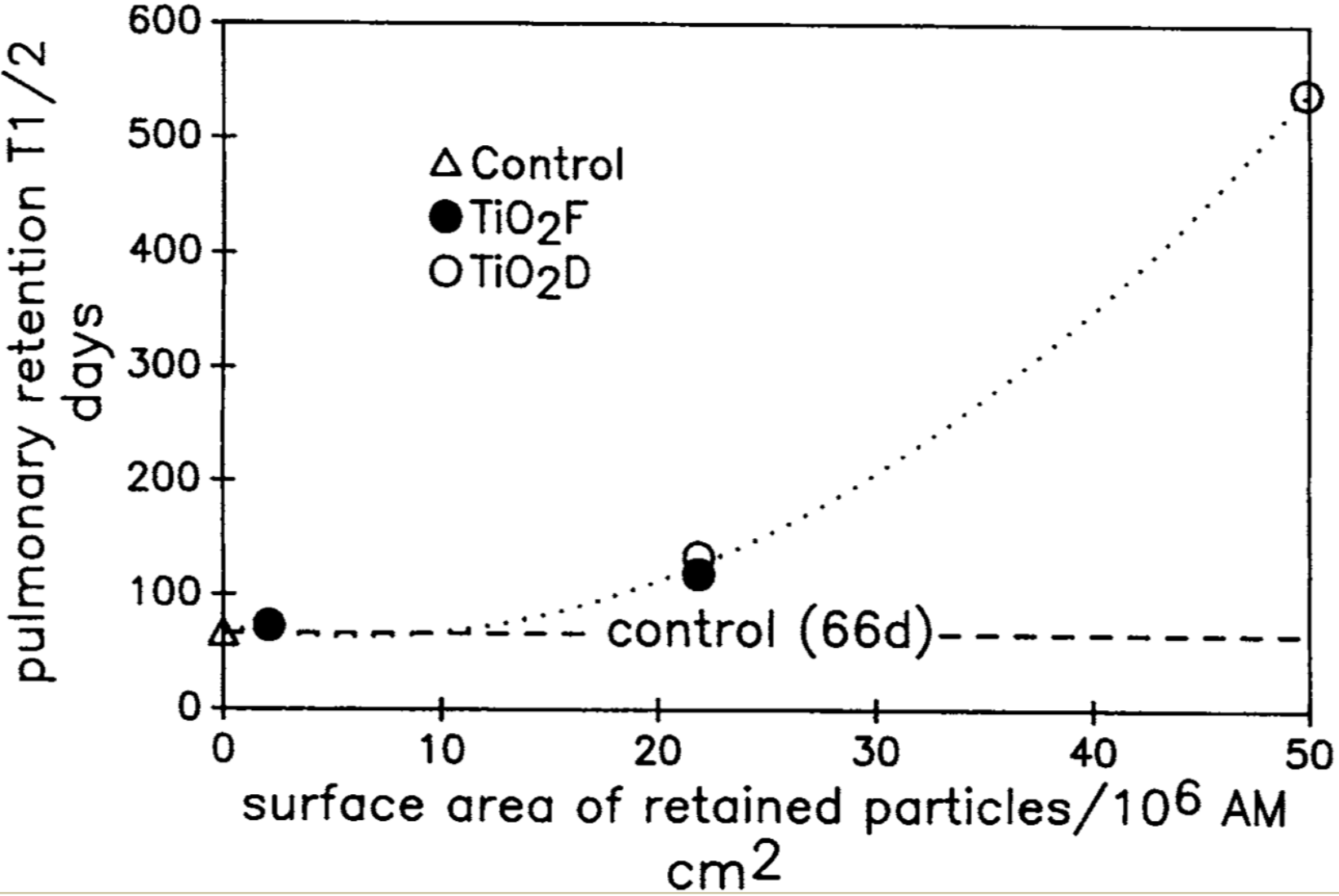
TiO_2 ultrafine (25 nm)	**99.8**	**26** *(768)*	**2.6** *(77)*	**49.9**	**5420**	**8.2***
Cristobalite	**~20**	**7.6** *(24)*	**0.76** *(2.4)*	**2.4**		**28.8***

***Significantly different from control**

(packing density volume)

Oberdörster et al, 1994

Correlation between surface area of TiO_2 particles phagocytized by AM and pulmonary retention half-time of inhaled polystyrene test particles



From: Oberdörster et al., 1994 (EHP)

Surface Reactivity as Dose-Metric,

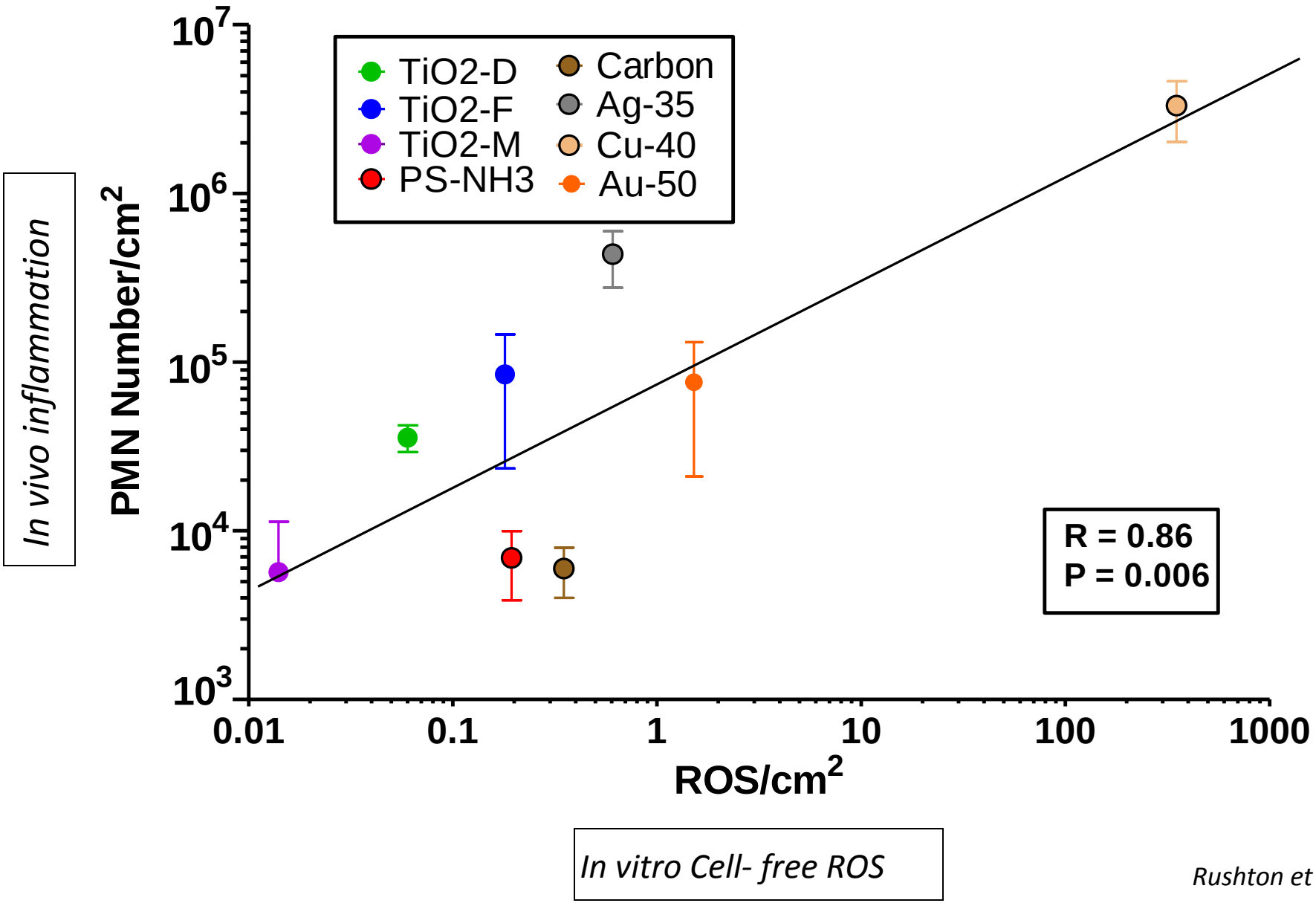
***e.g.*, ROS inducing potential to determine response per unit particle surface area**

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 in using cell-free assay, but only for ***Hazard Identification***

[Bello et al., 2009; Rushton et al., 2010]

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



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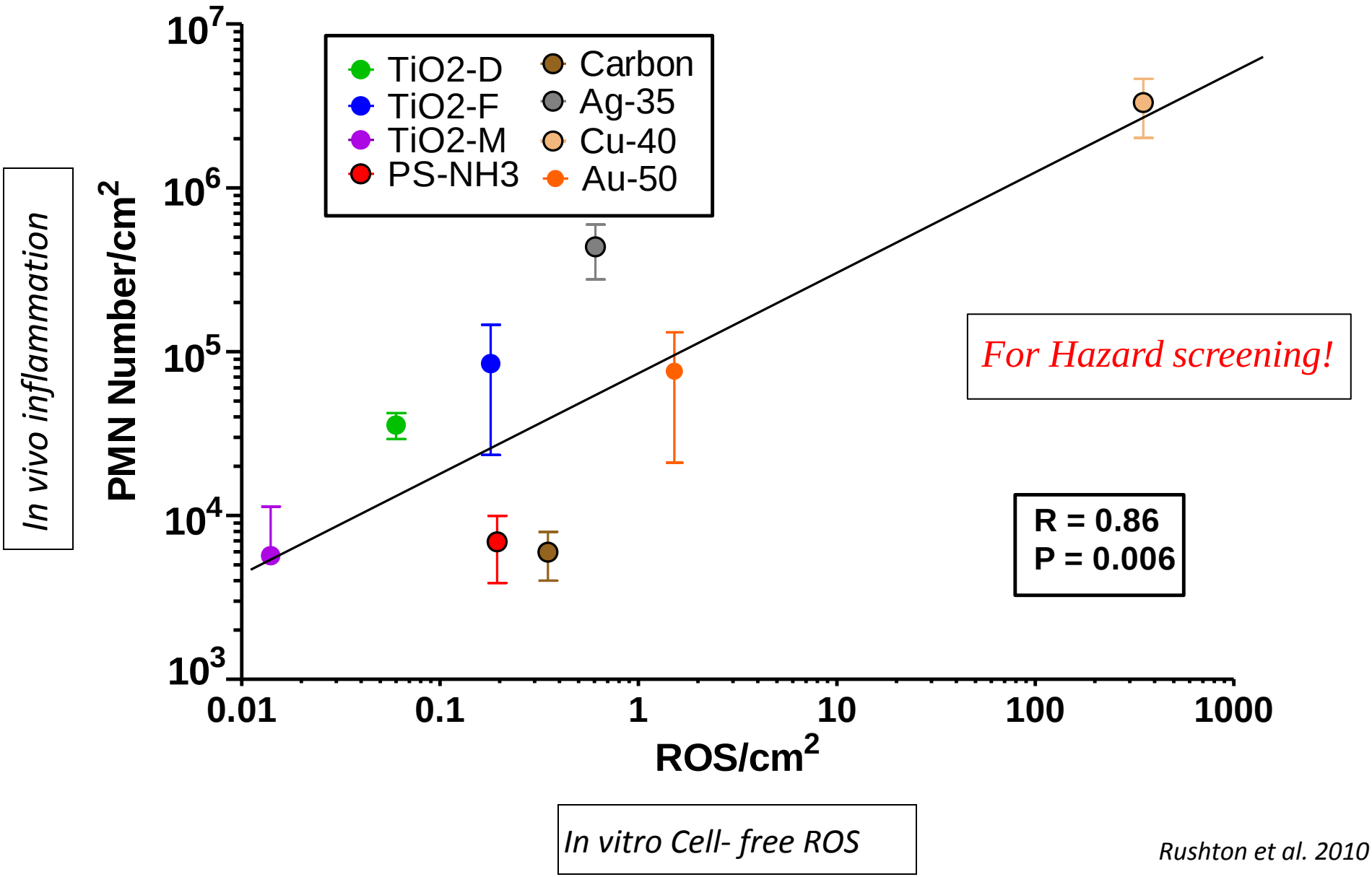
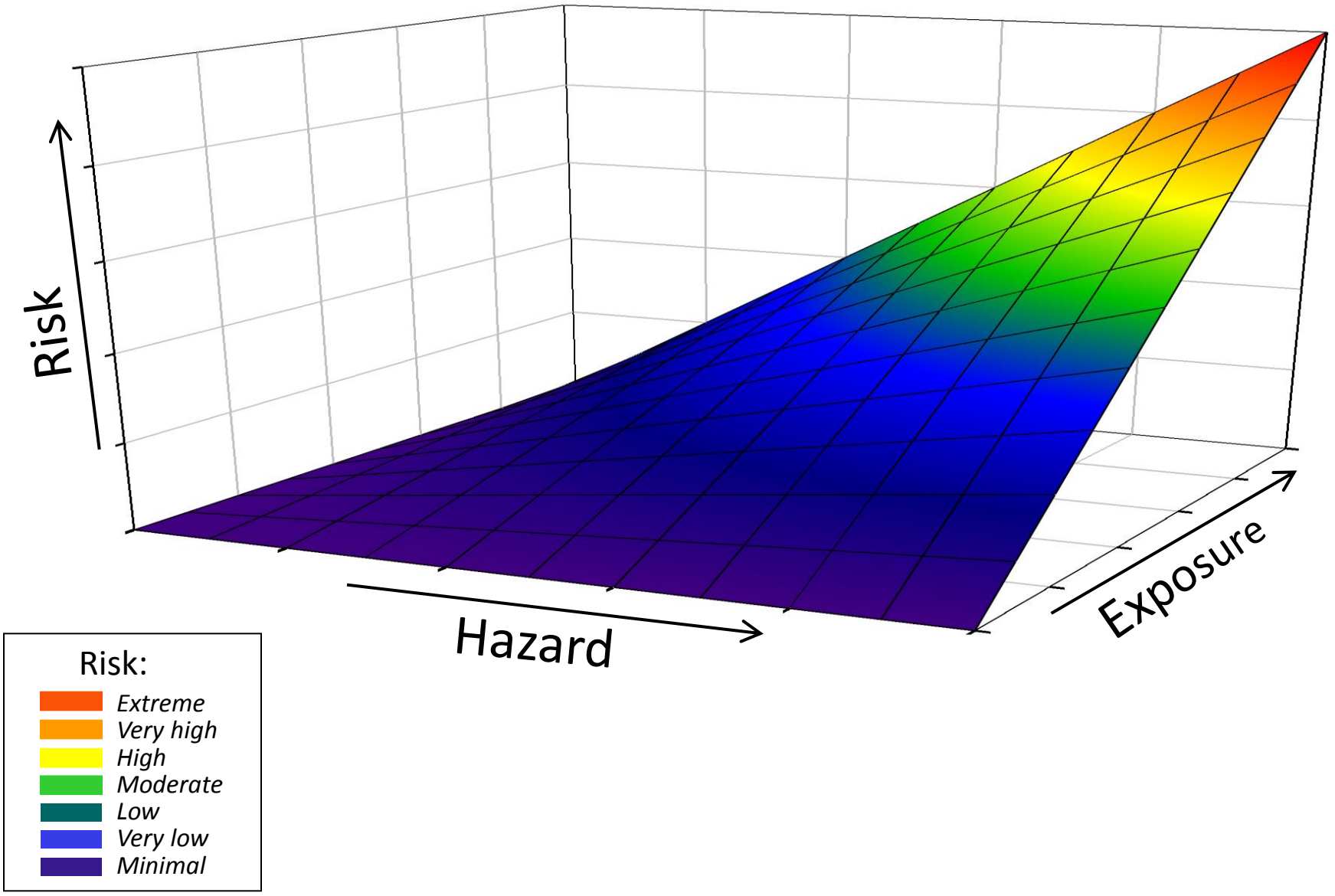


Rushton et al. 2010

Risk = f (hazard; exposure)



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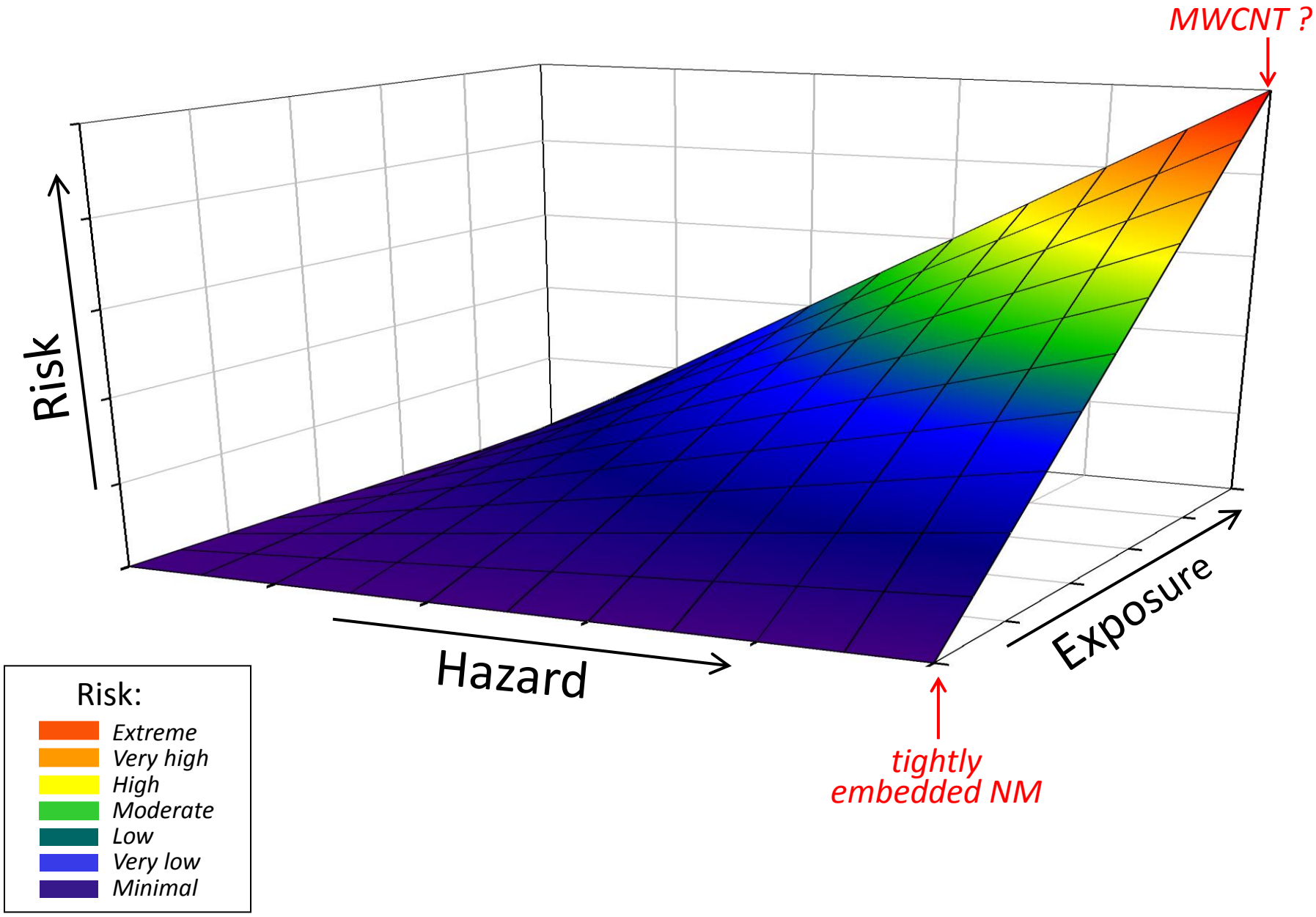

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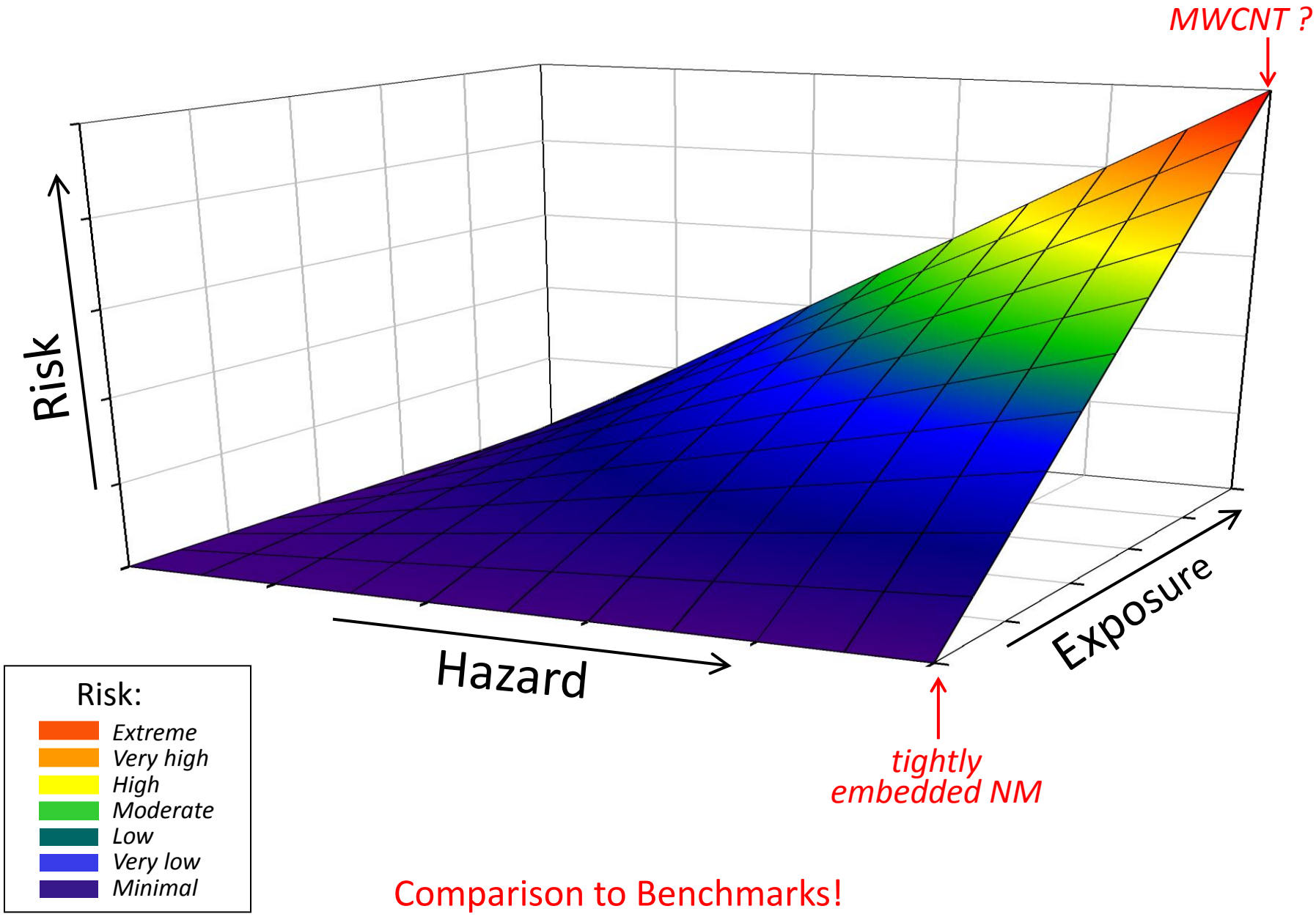
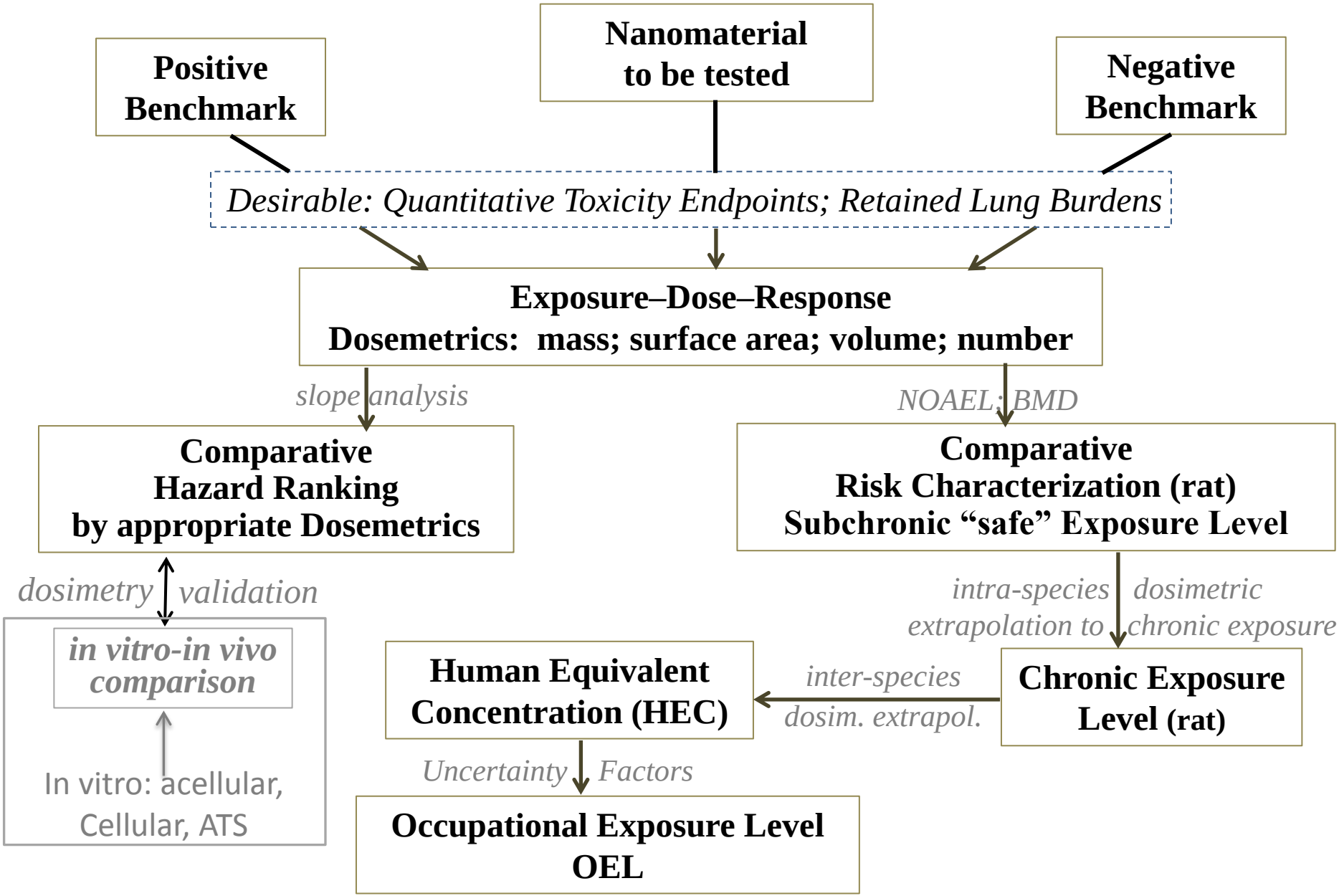


Comparison to Benchmarks!

Approach for Comparative Hazard and Risk Characterization of Inhaled Nano-Particles Based on Subchronic (3 months) Rat Inhalation Studies



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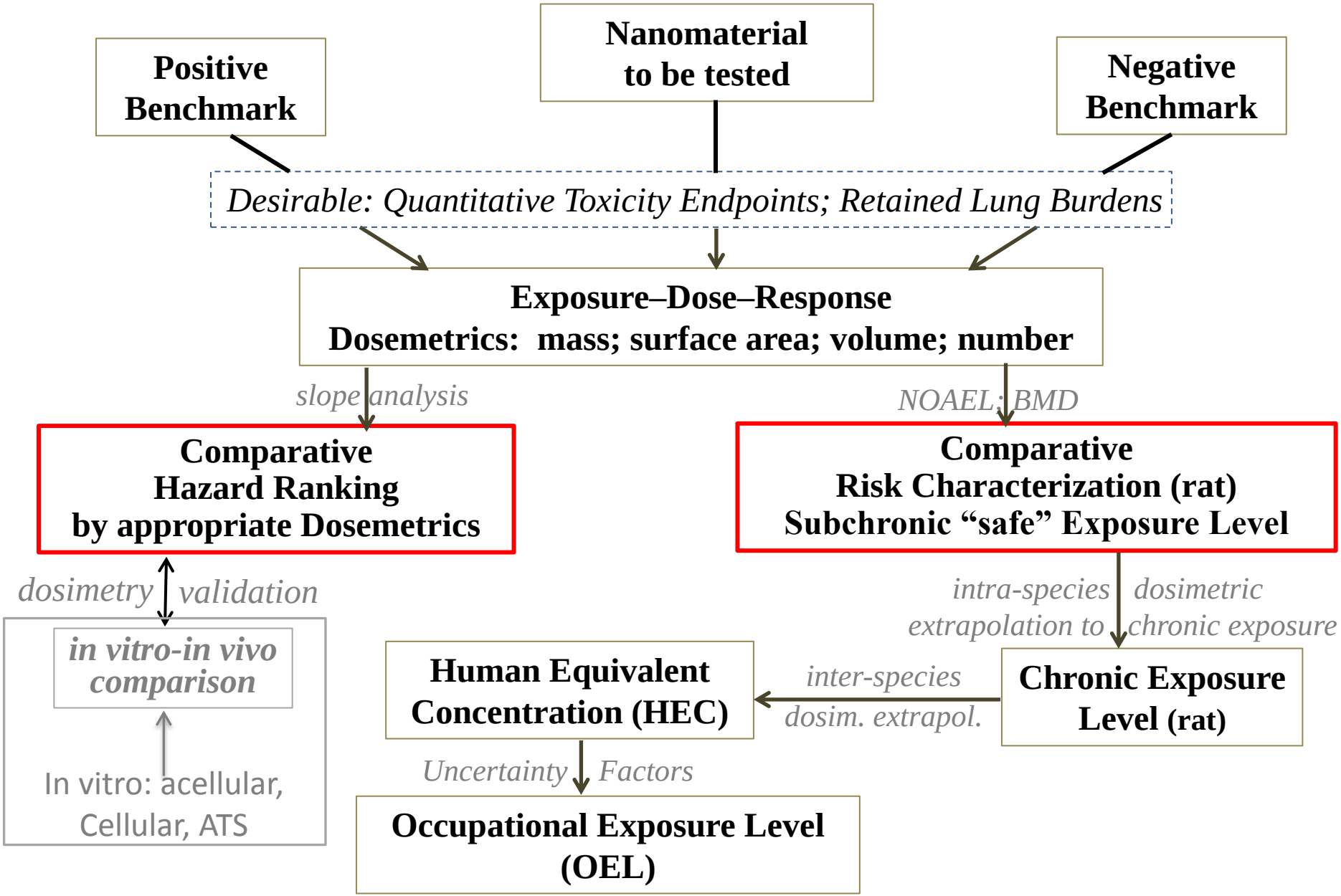

Case Study

90-Day Rat Inhalation Studies with MWCNT and CNF, Exposure-Dose-Response Comparison

	Ma-Hock et al. (2009)	Pauluhn (2010)	Kasai et al. (2014)	DeLorme et al. (2012)
Material	**MWCNT** (Nanocyl NC7000)	**MWCNT** (Baytubes)	**MWCNT** (MWNT-7)	**CNF** (VGCF-H)
Characterization				
Length/diameter, nm	**100-10,000 /5-15**	**200-1000/10**	**2000-5700/40-90**	**1000-14000/40 -350**
Impurities	**9.6% Al; <0.2% Co**	**~0.5% Co**	**0.2 - 0.4%**	**0.003% Fe**
BET surf area, m^2/g	**250 – 300**	**255**	**24-28**	**13.8**
Packing dens, g/cm^3	**0.043**	**0.11 - 0.31**	**0.007**	**0.077**
Exposure				
Conctr, mg/m^3	**0; 0.1; 0.5; 2.5** *(NO)*	**0, 0.1; 0.4; 1.5; 6** *(NO)*	**0; 0.2; 1; 5** *(WB)*	**0.54; 2.5; 25** *(NO)*
Ret. Lung Burden	**No**	**Yes**	**Yes**	**No**
Response				
Lung weight *(90 days)*	**+ 1%; + 23%; + 81%**	**+0; + 12; +27; +61%**	**+5; +17; +30%**	**-2; +8; +22**
BAL–PMN *(90 days)*	**Not reported**	**~0.5; 3.8; 13; 19%**	**0.6; 13.5; 45.7%**	**1.4; 2.7; 11%**
Evaluation				
NOAEL	**No**	**100 $\mu g/m^3$**	**No**	**540 $\mu g/m^3$**
LOAEL	**100 $\mu g/m^3$**	**400 $\mu g/m^3$**	**200 $\mu g/m^3$**	**2500 $\mu g/m^3$**

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Comparing MWCNT and CNF results with

subchronic rat inhalation studies of two Benchmark compounds:

ultrafine carbon black — *negative*

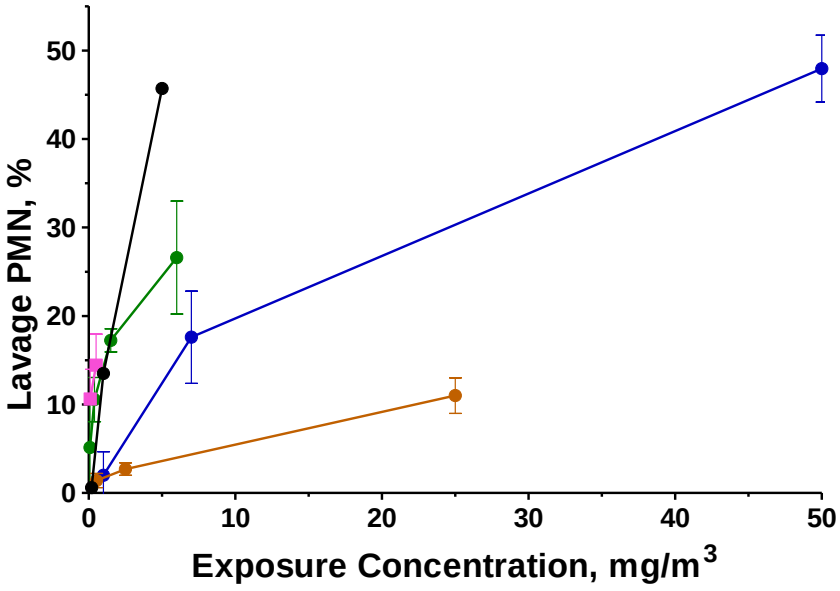
nickel subsulfide — *positive*

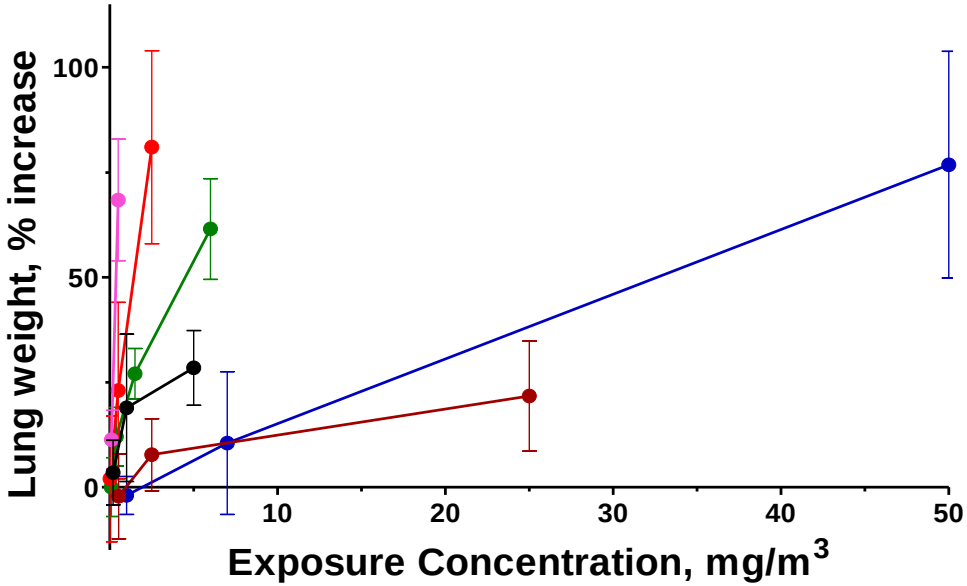
Benchmark particles

Exposure-Response Relationships

3-Month Inhalation Studies in Rats with MWCNT, CNF, CB and Ni_3S_2

—Endpoints: Lung lavage neutrophils and lung weight —





● - MWCNT (*Pauluhn, 2010*); ● - MWCNT (*Ma-Hock et al., 2009*);

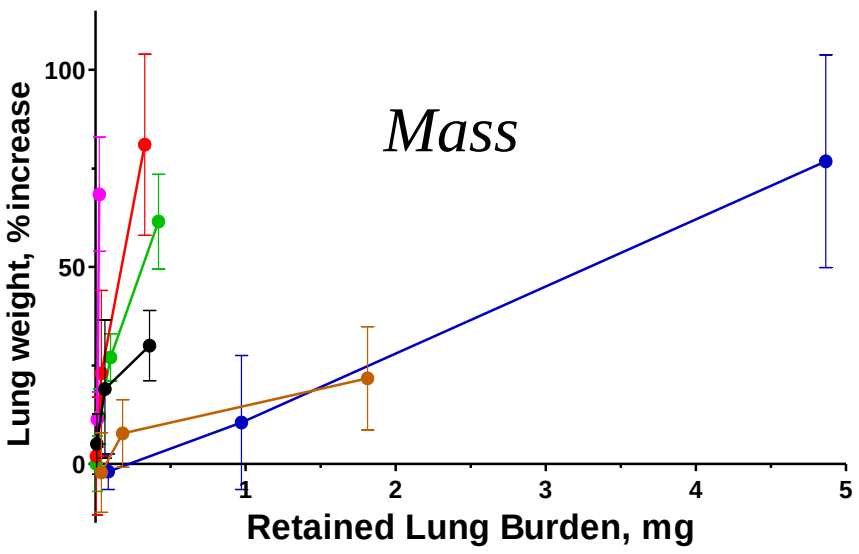
● - Ni_3S_2 *(Oberdörster et al., unpublished);* ● - **CNF** (*DeLorme et al., 2012*);

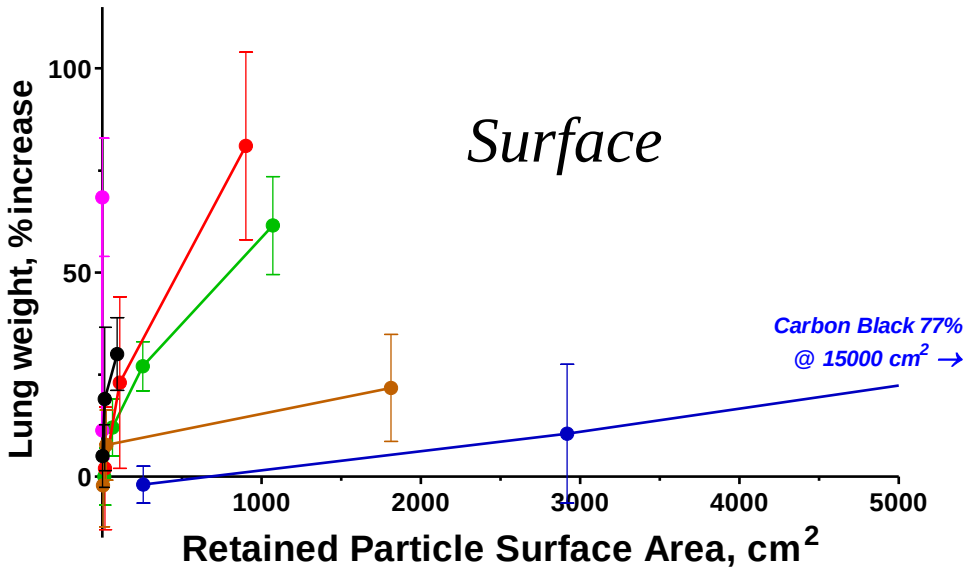
● - CB (*Elder et al., 2005*); ● - MWCNT *(Kasai et al., 2014)*

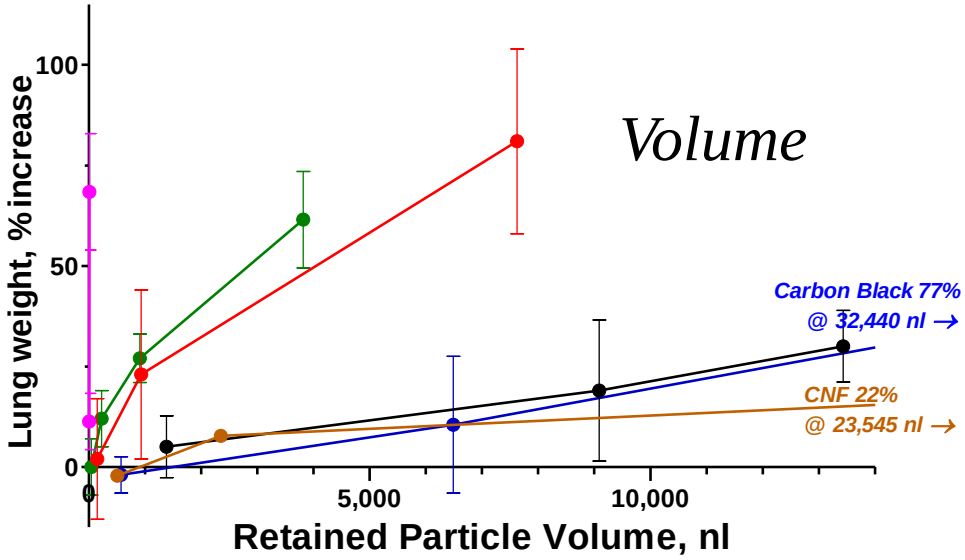
Dose-Response relationships

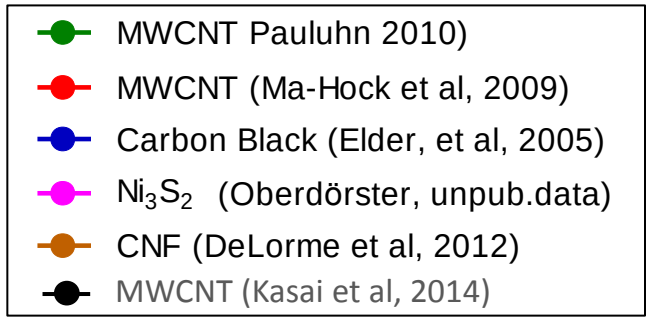
3-month inhalation studies in rats with MWCNT, CNF, CB and Ni_3S_2

– Lung weight dose-responses based on retained lung burden expressed as mass, surface area and volume –









Hazard Ranking of Different (Nano)-Materials Based on Different Metrics and Steepest Slope of Exposure-Dose-Response Relationships from Subchronic Rat Inhalation Studies (endpoint: lungweight increase)

Metric	Ranking
Exposure Conc. **:**	**CNF = CB < MWCNT-K = MWCNT-P = MWCNT-MH < Ni_3S_2**
Retained Lung Burden:	
Mass:	**CNF = CB < MWCNT-P = MWCNT-K = MWCNT-MH < Ni_3S_2**
Surface area:	**CB < CNF = MWCNT-P = MWCNT-MH<MWCNT-K < Ni_3S_2**
Volume (bulk dens)**:**	**CB < CNF = MWCNT-K < MWCNT-MH = MWCNT-P < Ni_3S_2**

Hazard Ranking of MWCNT and CNF against Benchmark Materials based on retained Particle Surface Area and Steepest Slope of Dose-Response Relationships from Subchronic Rat Inhalation Studies (endpoint: **lungweight** increase)

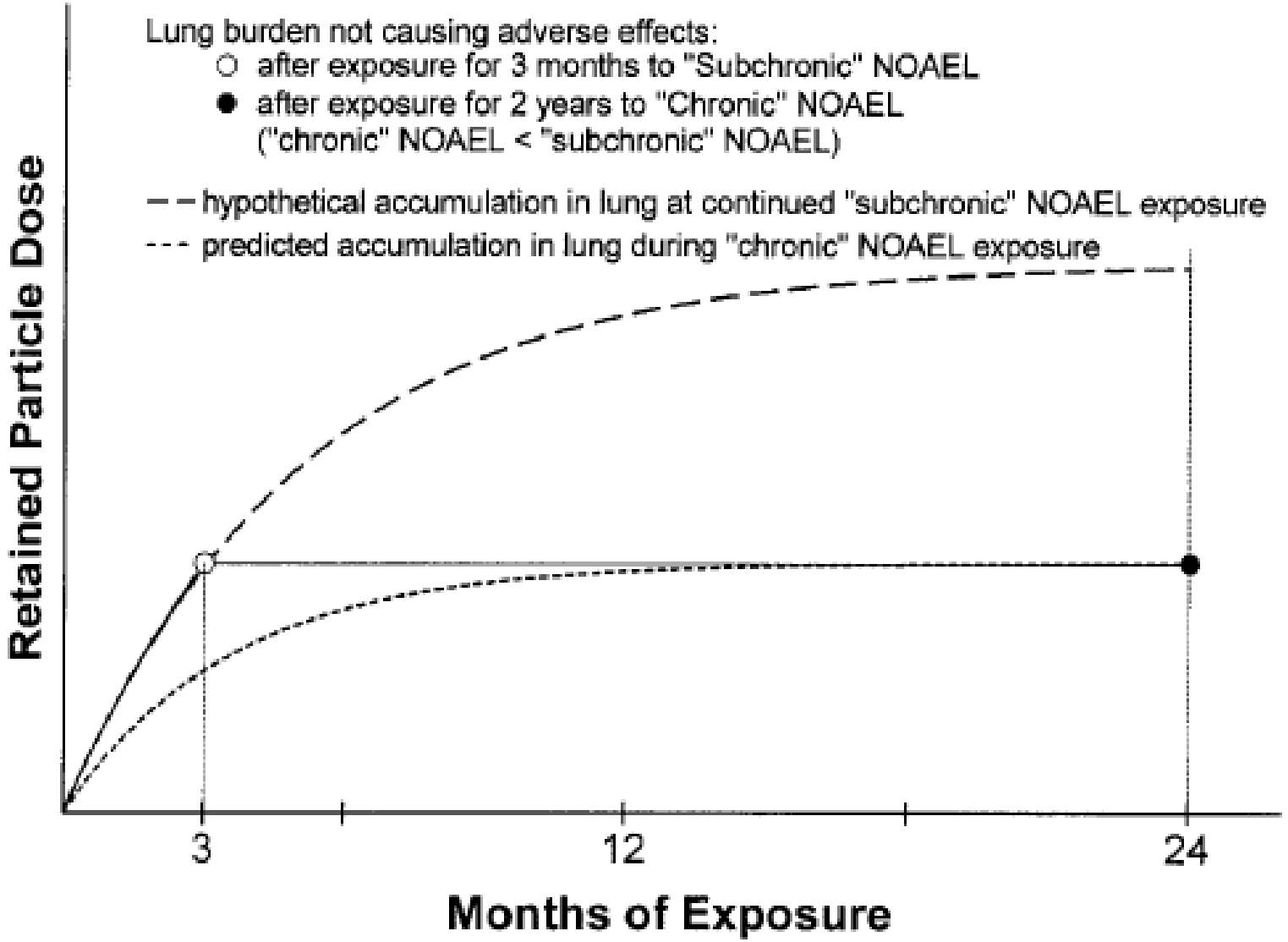
Three Hazard Groupings:

Low: ***CB*** → ***< 0.3 % lungwt. incr./cm²***

Medium: ***MWCNT; CNF*** → ***0.3 – 1.5 % lungwt. incr./cm²***

High: **Ni_3S_2** → **> 1.5% lungwt. incr./cm²**

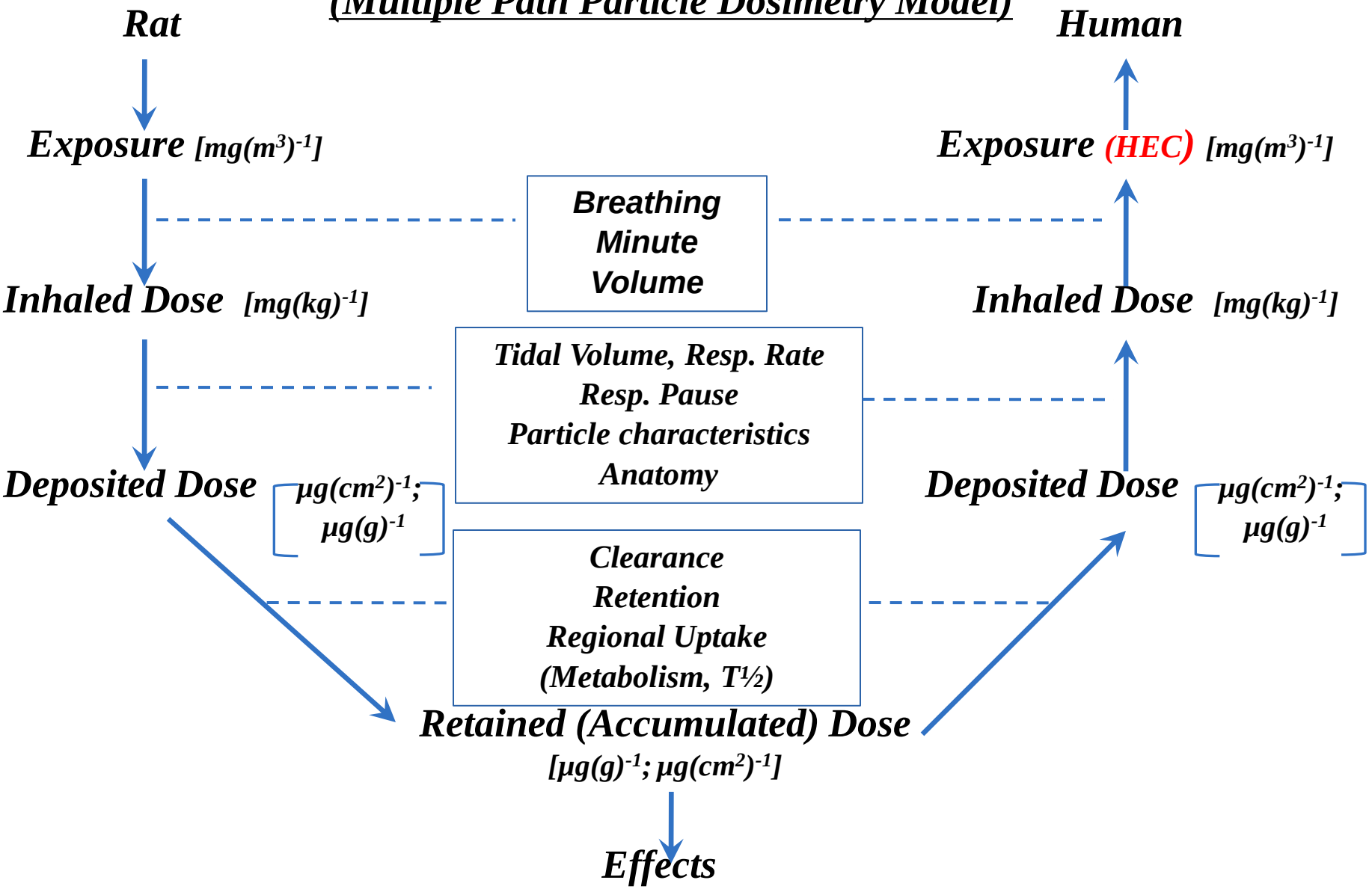
Estimation of Chronic NOAEL from Subchronic Rodent Study using MPPD Model



From: Oberdörster, 2002

Dosimetric Extrapolation of Inhaled Particles from Rats to Humans

(Multiple Path Particle Dosimetry Model)



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

Estimation of HEC through BMD analysis of subchronic rat studies by using rat responses as 1 St. Dev. or as 10 % above control (BMCL). Endpoint: LUNG WEIGHT Increase

Material	Rat					Human			
	subchronic			chronic		chronic			
	BMDL µg/lung	**BMCL µg/m³**	Daily Depos.Dose µg/6 hr (depos.fract)	Daily Depos.Dose µg/6 hr	**BMCL** µg/m³	BMDL µg/lung	BMDL µg/alv. compt.	Daily Depos.dose µg/8 hr (depos.fract)	**HEC µg/m³**
MWCNT(P)	**16.8**	**260**	**0.39** (0.022)	**0.23**	**136**	**14,000**	**8,820**	**36.2** (0.073)	**50**
MWCNT(M)	**4.6**	**72**	**0.11** (0.019)	**0.06**	**41**	**3,830**	**2,413**	**9.91** (0.062)	**16.3**
CNF	**97**	**1450**	**2.5** (0.022)	**1.5**	**865**	**8,083**	**5,092**	**20.9** (0.064)	**33.2**
CB	**555**	**4180**	**13.0** (0.024)	**7.7**	**4160**	**462,500**	**291,375**	**1,196** (0.076)	**1600**
Ni_3S_2	**2.98**	**25**	**0.07** (0.034)	**0.04**	**15.3**	**2,483**	**1,564**	**6.42** (0.109)	**6.0**

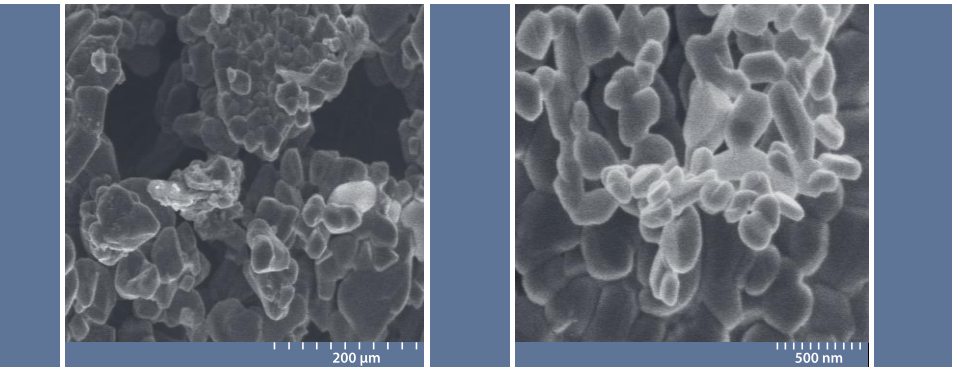
MWCNT and CNF cannot be categorized as PSP

Human breathing conditions: light exercise; TV: 1024 ml; BrFreq: 20 min^{-1}; 8 hr; oro-nasal breathing

CURRENT INTELLIGENCE BULLETIN 63

2011

Occupational Exposure to Titanium Dioxide



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

REL: Fine: 2.5 mg/m³
Nano: 300 µg/m³

2013

REL: 1 µg/m³

CHALLENGES FOR ESTABLISHING Occupational Exposure Levels *(OEL)* FOR CNT/CNF:

***Workplace monitoring*: 1 µg/m^3; distinguishable from background?**

***One generic OEL for all*: Are all CNTs and CNFs toxicologically of equal potency?**

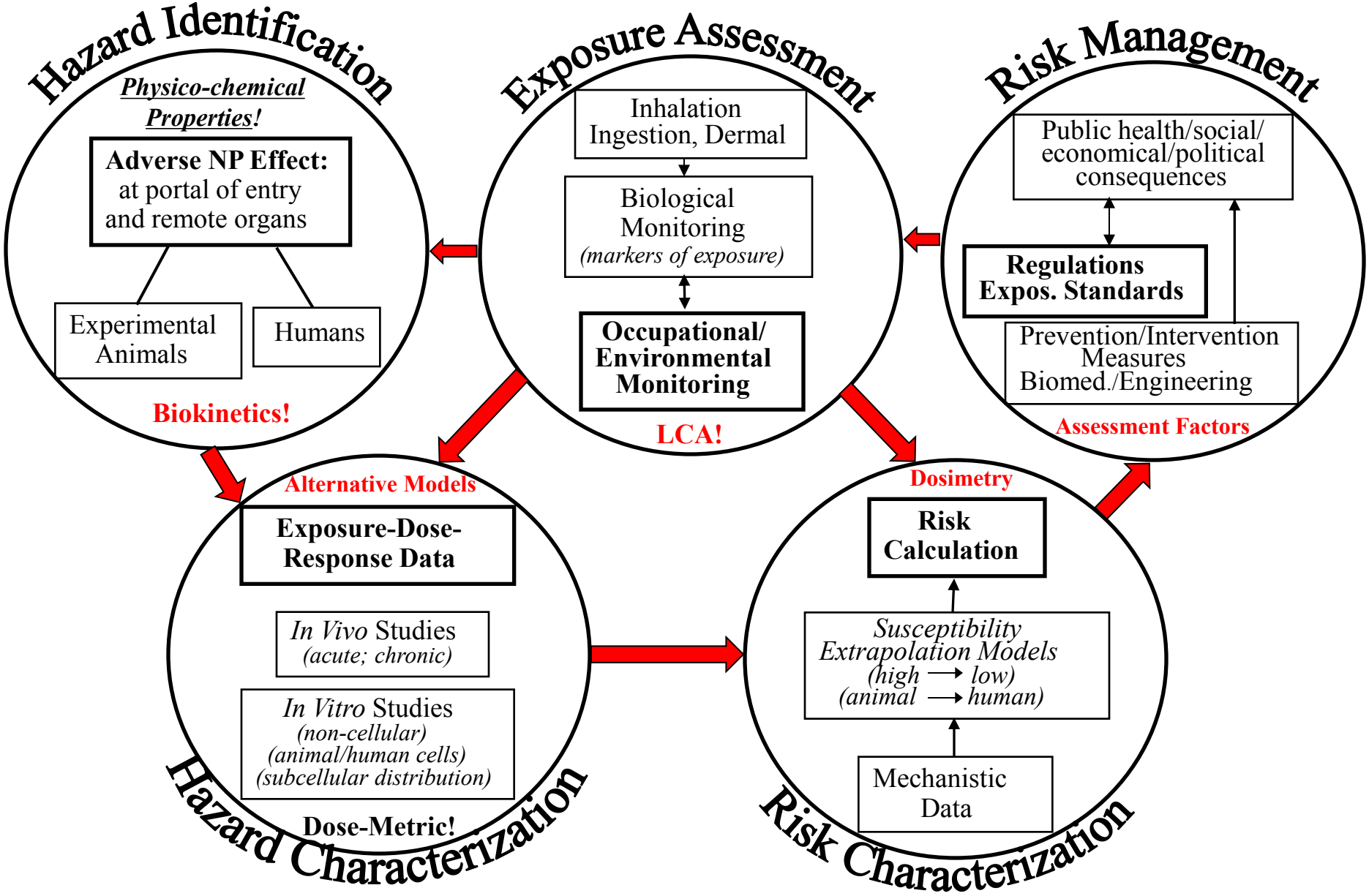
In addition to dimension, surface modification or functionalization, tangles, straightness, level of impurities, surface defects are known to alter toxicity:

MWCNT-x ≠ MWCNT-y

However, with no convincing data to the contrary, it is prudent to treat airborne CNTs/CNFs as hazardous

Needed: Results of chronic rodent inhalation study

Risk Assessment and Risk Management Paradigm For Engineered Nanoparticles (NPs)



Desirable as basis for testing and for regulatory hazard and risk characterization:

Establishing toxicologically well defined Benchmark Materials as tool for classification