NANOPARTICLE SURFACE ACTIVITY:

UNDERSTANDING, MEASURING, AND INTEGRATING IT INTO DOSIMETRY

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The CHALLENGE

THE VIAL

THE FILTER

Outline

- Surface in Inhalation Dosimetry
  - Surface Area & Activity
  - Bench top Technologies and Options
- Surface in ENM Exposure Assessment
  - Gaps & Needs
- Near Real-time monitoring of SAr and SAc

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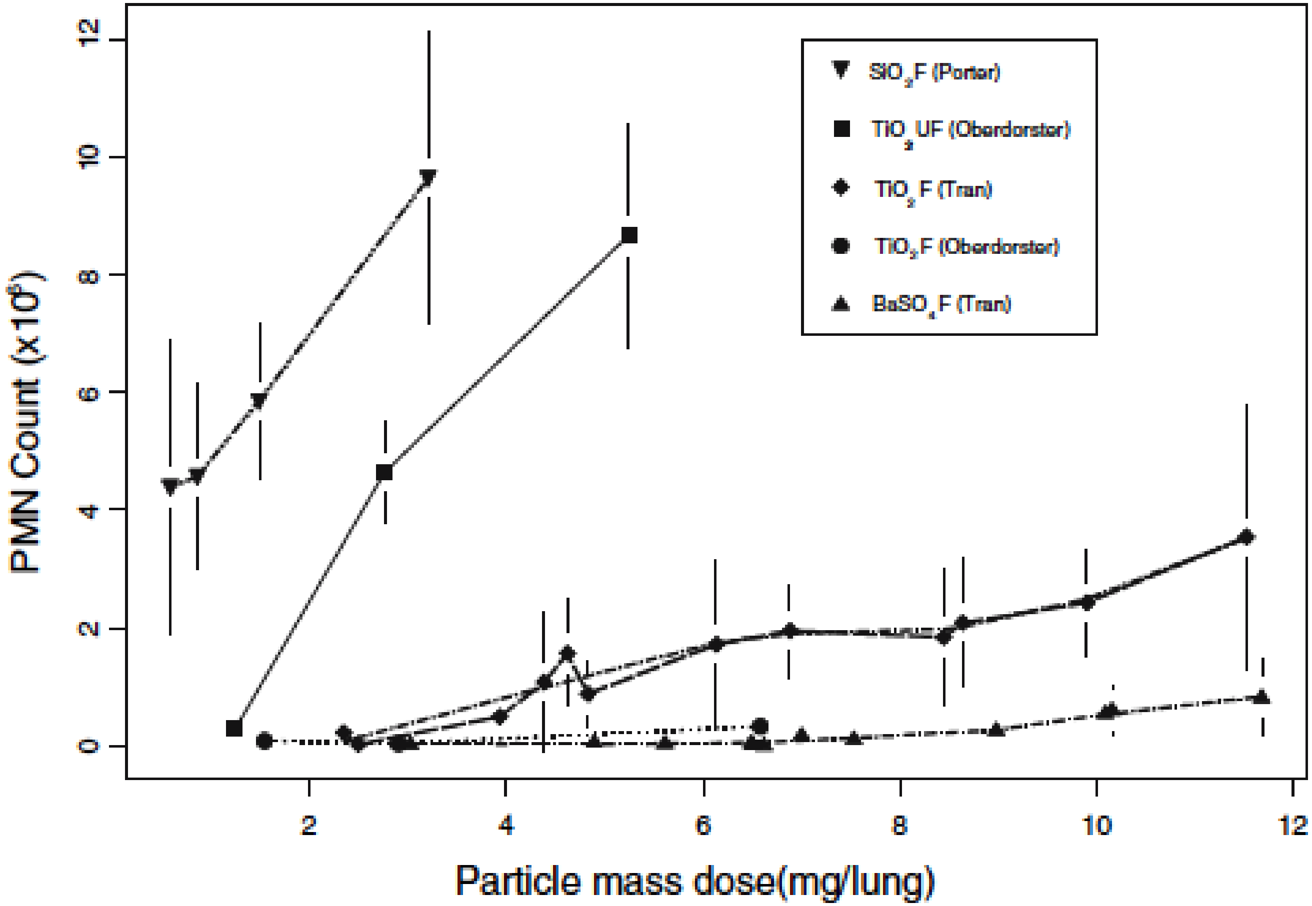
Dose Metrics: A Historical Perspective

MASS
(ug/m3)

RESPIRABLE
FIBERS
(f/m3)

Viable & Total
Microorganisms
(#/m3)

Departing from Mass (OR NOT?)



Maynard & Kuempel 2005

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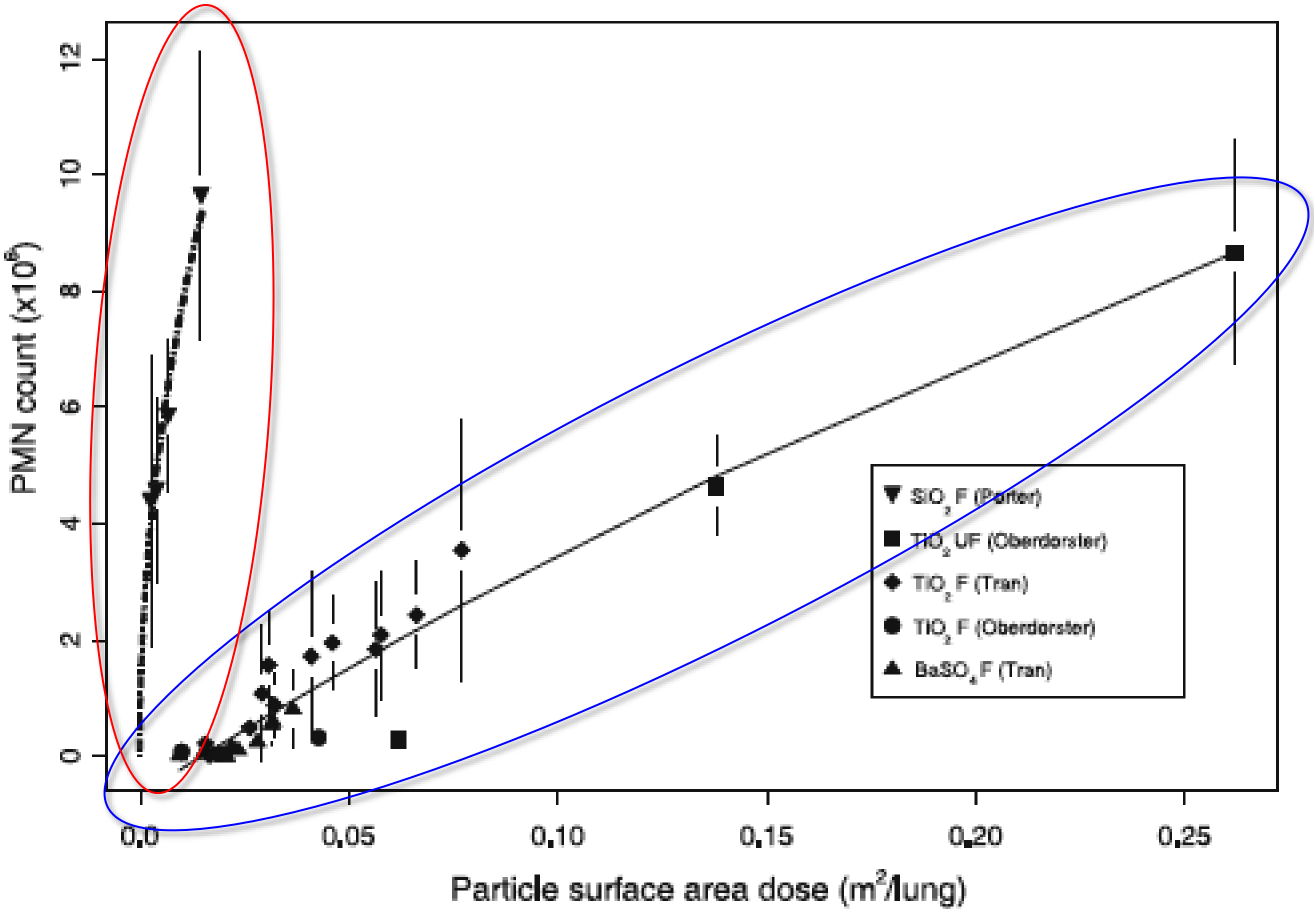
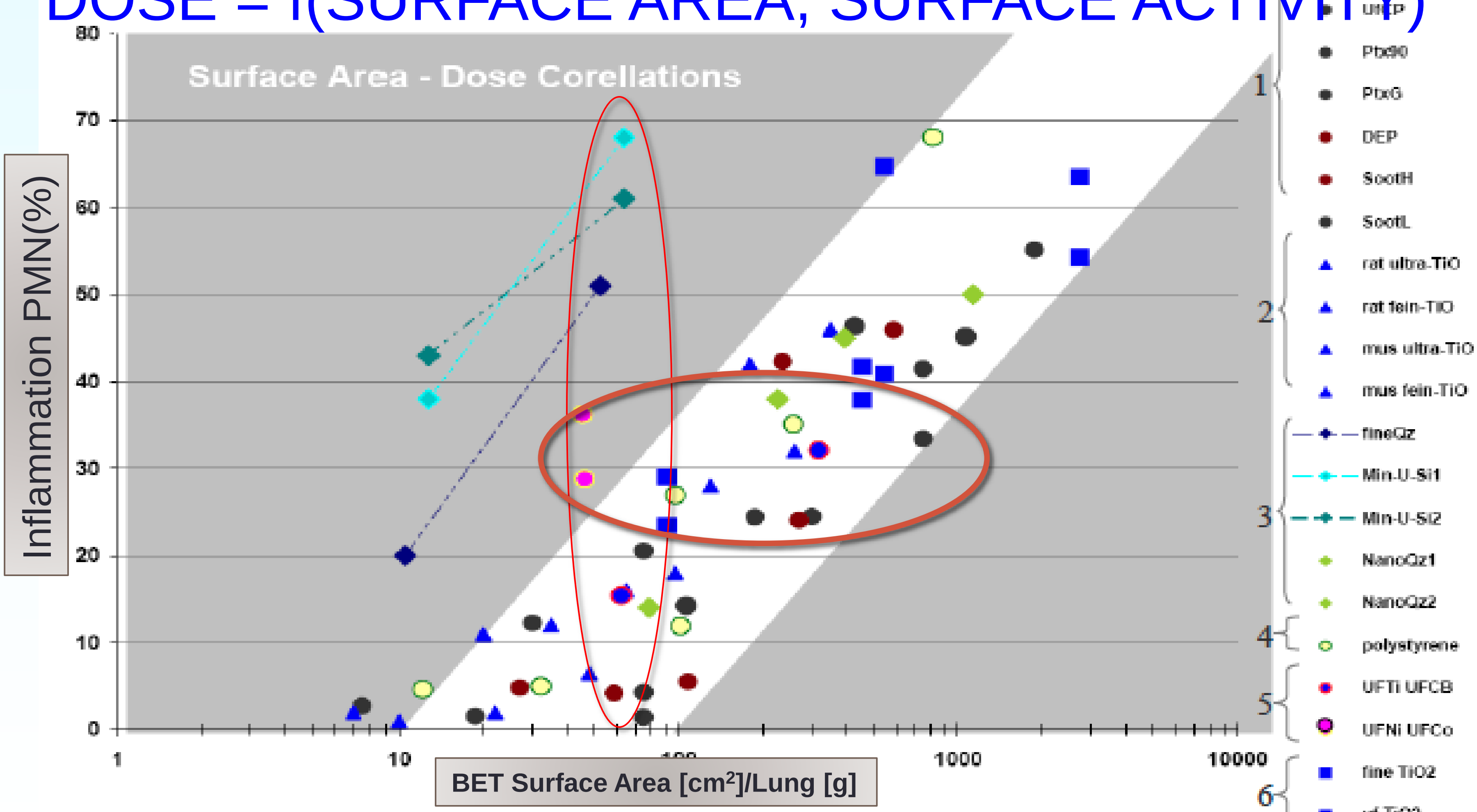
SURFACE AREA METRIC



Maynard & Kuempel 2005

Surface Area as a Dose Metric

DOSE = f(SURFACE AREA, SURFACE ACTIVITY)



[1]Stoeger 2006, [2]Oberdörster 2005, [3]Warheit 2006, [4]Brown 2001, [5]Dick 2003, [6]Höhr 2002

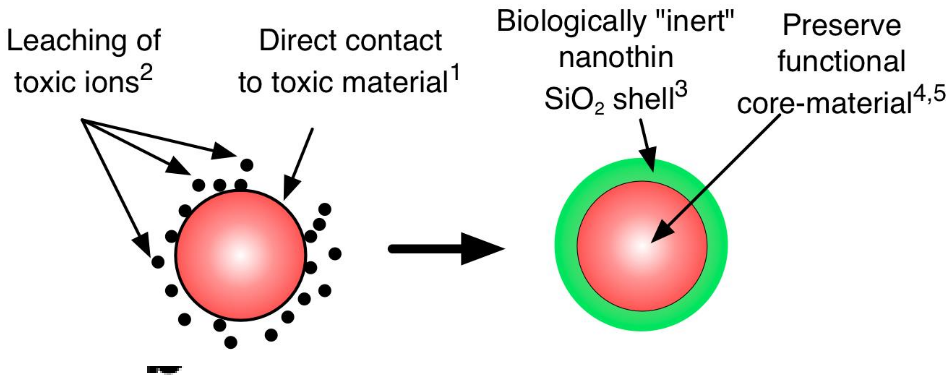
Physicochemical & Morphological Properties Influencing Toxicity

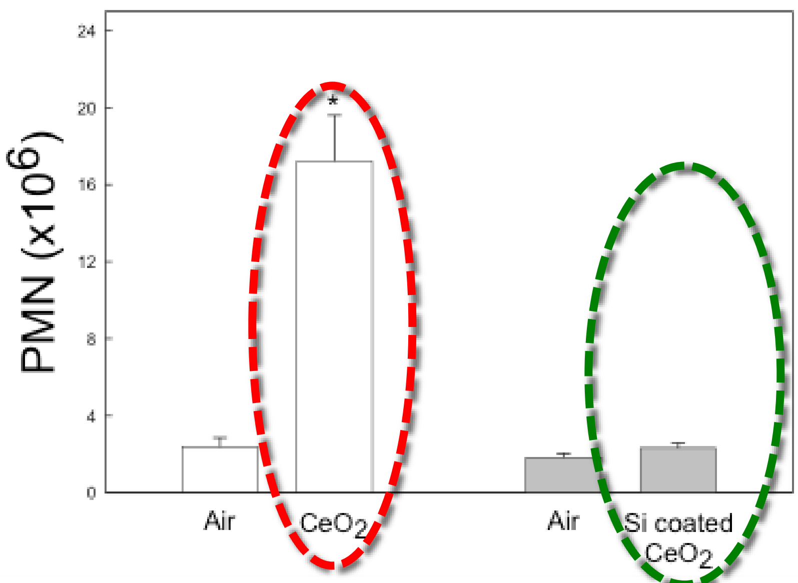
- Size Distribution
- *Surface Area*
- *Surface Chemistry*
- Surface Charge
- Bulk Chemical Composition
- *Metals & Impurities*
- Morphology
- *Crystalinity*
- Biopersistence
- Metal Leaching….

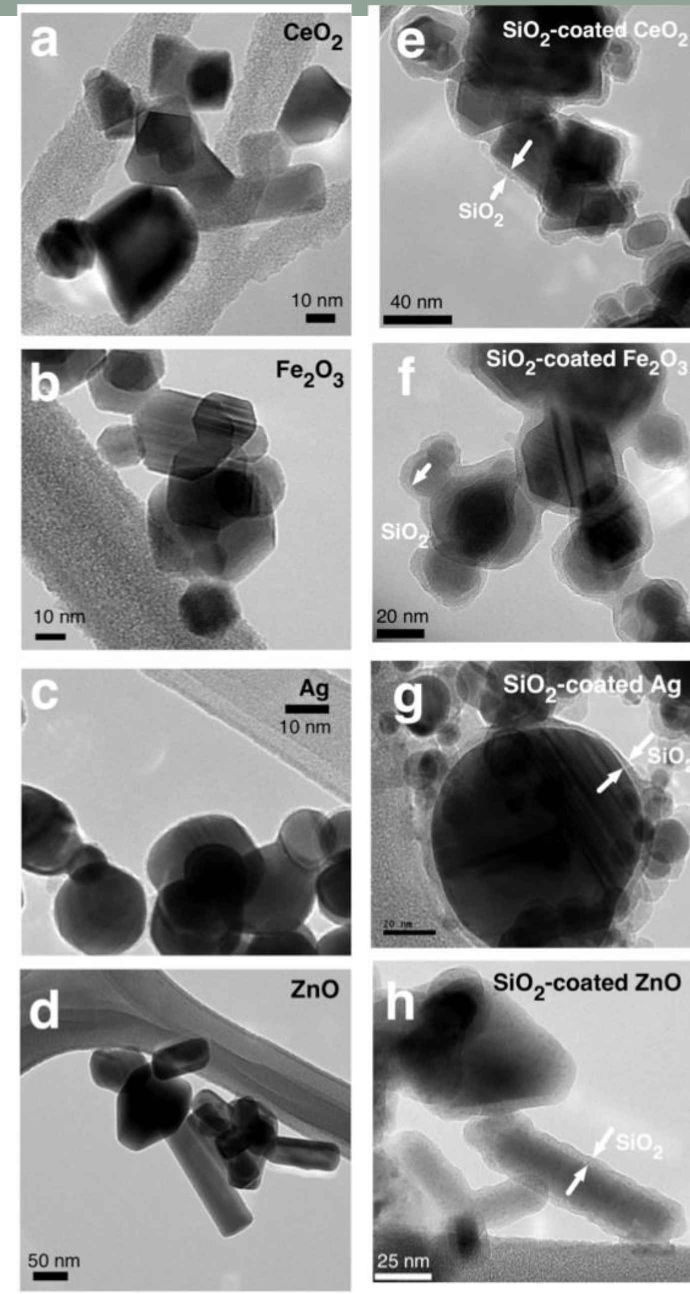
28 Properties in all (ICON 2007)

- Surfaces are NOT equal!
- Multiple parameters related to surface properties (SP)!
- How to measure these surface properties?
- How do these measures relate to biology/toxicology?

Next generation ENM: Safer-by design







(1) Sotiriou et al., Curr Opin Chem Eng **2011**, 1, 3 – 10
(2) Xia et al., ACS Nano **2011**, 5, 1223 – 1235
(3) Napierska et al., *Particle and Fibre Toxicology* **2010**, *7*,39
(4) Teleki et al., *Chem. Mater.* **2009,** *21,* 2094–2100
(5) Sotiriou et al., *Adv. Funct. Mater.* **2010,** *20,* 4250–4257

Demokritou, ES Nano 2013

Impurities & Bioactivity

Doping of SiO_2 NPs with MeOx NP or Me^{n+}

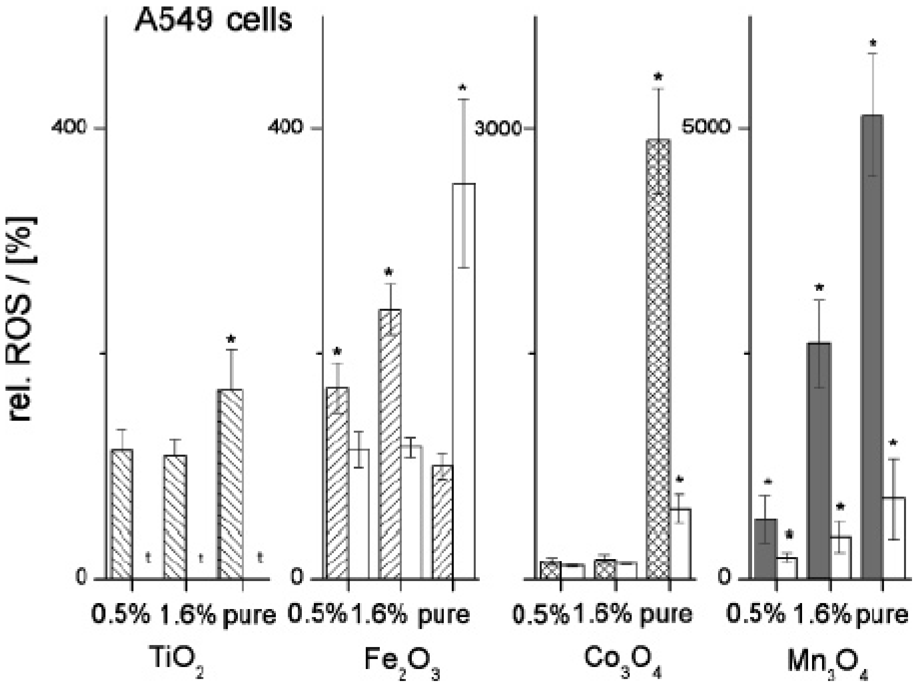


FIGURE 2. ROS concentrations in human lung epithelial cells after

Limbah et al ES&T 2007 41

CB +Fe2O3: Synergistic ROS generation

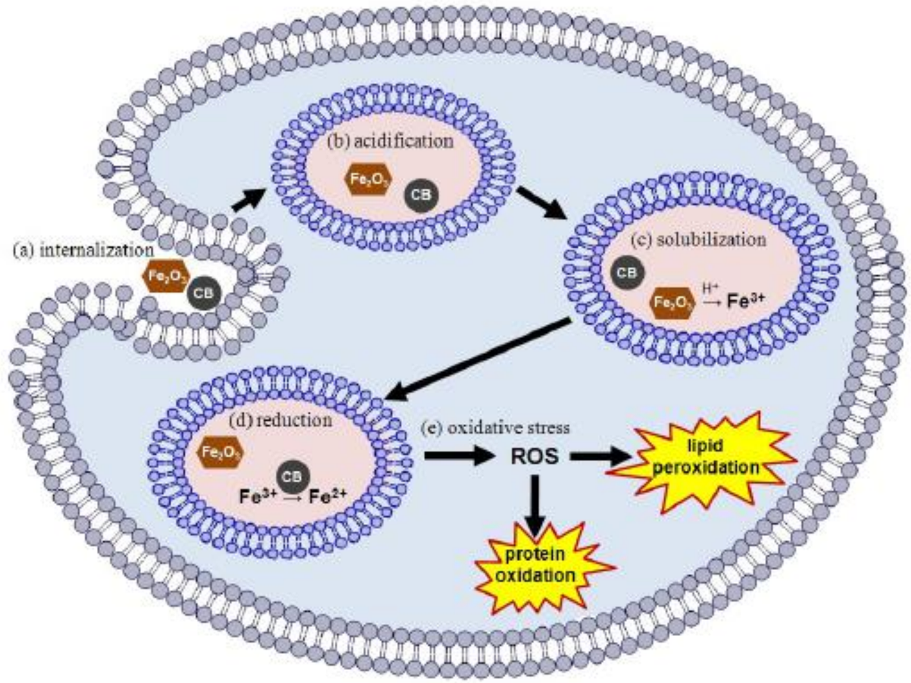


Figure 1
Illustration of events following concurrent endocytosis of both carbon black nanoparticles and Fe_2O_3 nanoparticles.

Guo et al PFT 2009

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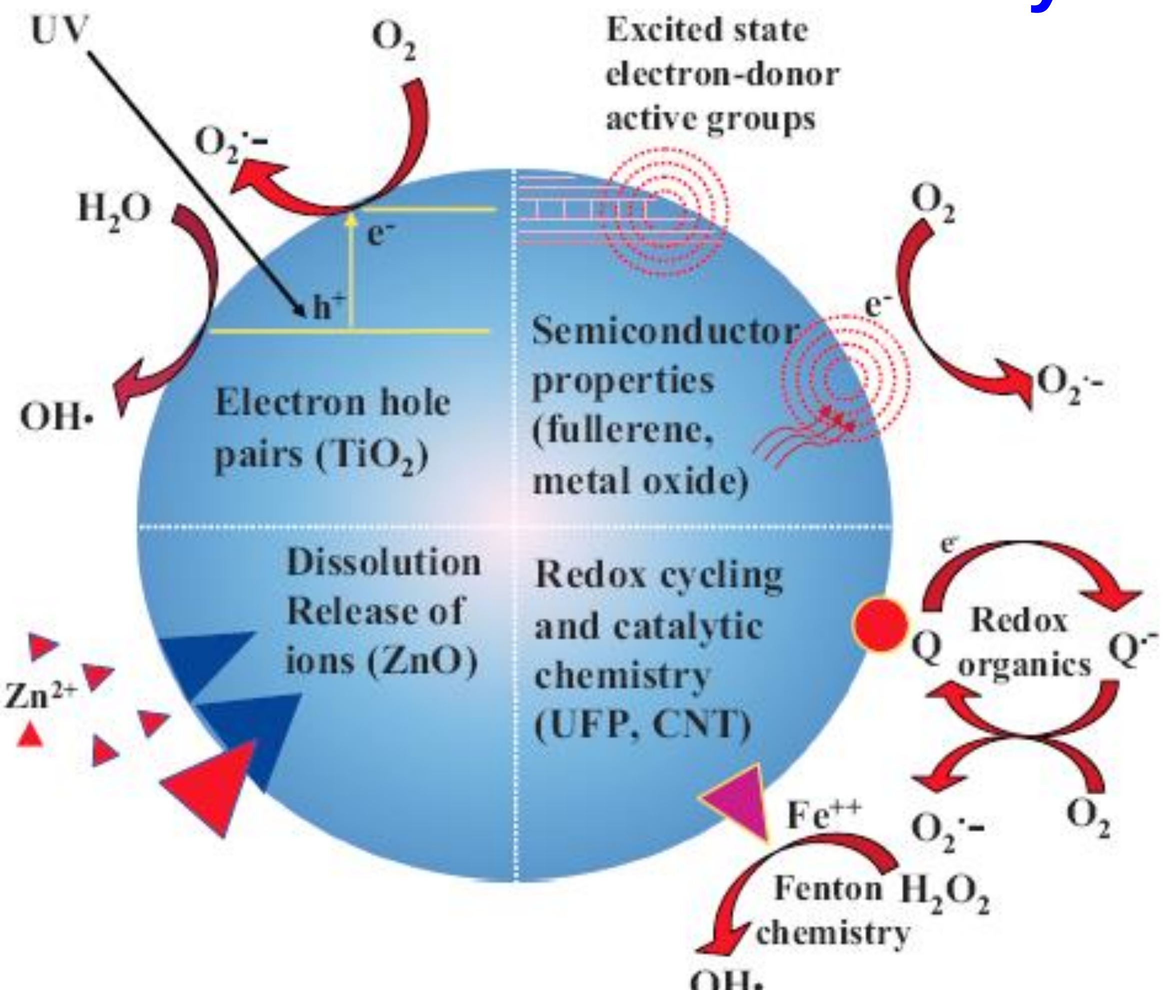
Oxidative stress – An important mechanism of NP toxicity



Nel et al. Science 311, 622 (2006)

Oxidative stress (OS) has been recognized in vivo and in vitro systems as one such major pathway and is being explored for ENM toxicity screening purposes (Nel *et al.* 2006; Xia *et al.* 2006; Borm *et al.* 2007; Ayres *et al.* 2008; Rogers *et al.* 2008; Bello *et al.* 2009; Lu *et al.* 2009; Meng *et al.* 2009).

Examples illustrate the importance of material composition, electronic structure, bonded surface species (e.g., metal-containing), surface coatings (active or passive), and solubility, including the contribution of surface species and coatings and interactions with other environmental factors

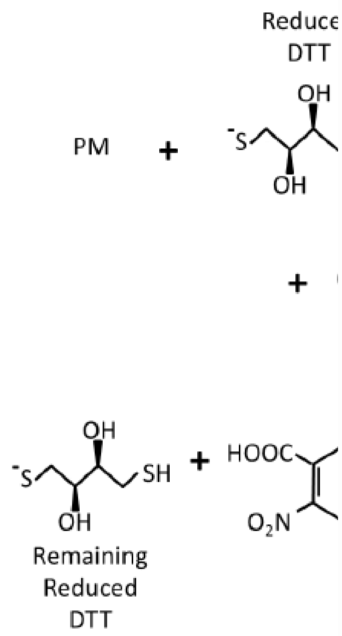
System		Markers (of Oxidative Stress)
In vitro	Cell free System	**Measure ROS generation** DTT assay DCFH-DA assay EPR/ESR
	Cellular System	Cell viability/Mitochondrial dysfunction ROS – DCFH-DA assay Activation of pro-inflammatory pathway Inflammatory factors, cytokine production Redox enzyme expression (HO-1, SOD) DNA damage/cell mutagenesis/proliferation Luciferase Reporter, Cyt C
In vivo	Inhalation Oral Administr. Skin irritation Aquatic animals	Similar to Cellular system GSH depletion Hematological, biochemical and pathologic change

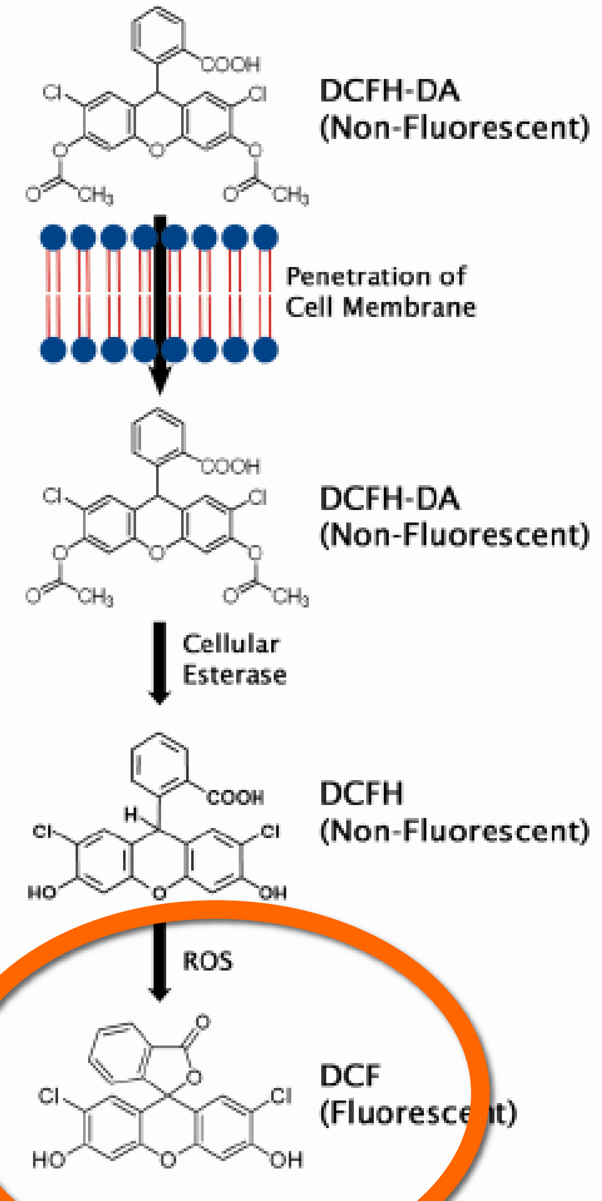
ROS - *Reactive oxygen species ;* DTT-*Dithiothreitol;* DCFH-DA-*Dichlorofluorescin diacetate;* HO-1; *Heme oxygenase-1; SOD-superoxide dismutase;* GSH-*Glutathione;* EPR-*Electron paramagnetic resonance*; *ESR-electron spin resonance*

Approaches

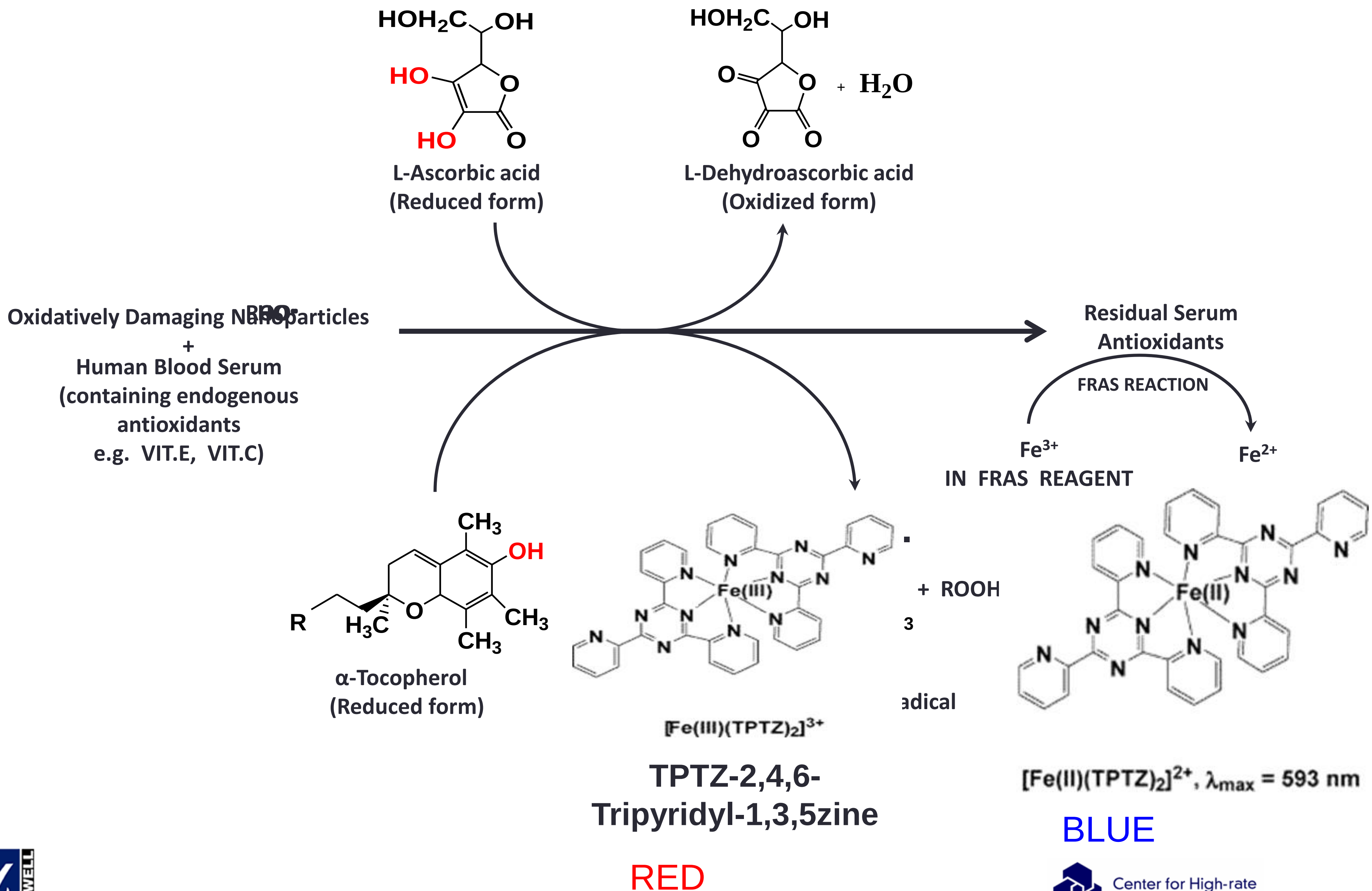
Acellular

- ESR/EPR
  - Spin trapping with select agents (e.g. DMPO)
- DTT
  - Colorimetric
- DCFH
  - Fluorescence (RO_2, RO, OH·, HOCl and ONOO but not $O_2^{\cdot-}$, and H_2O_2)
- FRAS
  - Human Serum - Colorimetric





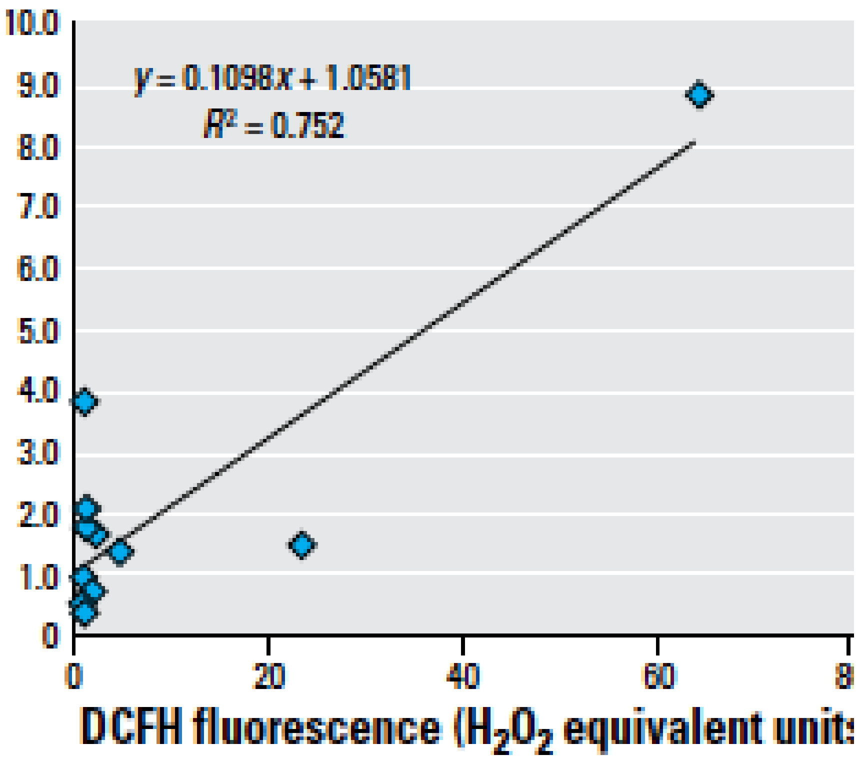
ANALYTICAL APPROACH TO DETERMINE THE DEGREE OF OXIDATIVE STRESS EXERTED BY NANOPARTICLES IN HUMAN BLOOD SERUM BY FERRIC REDUCTION ACTIVITY OF SERUM (FRAS ASSAY)



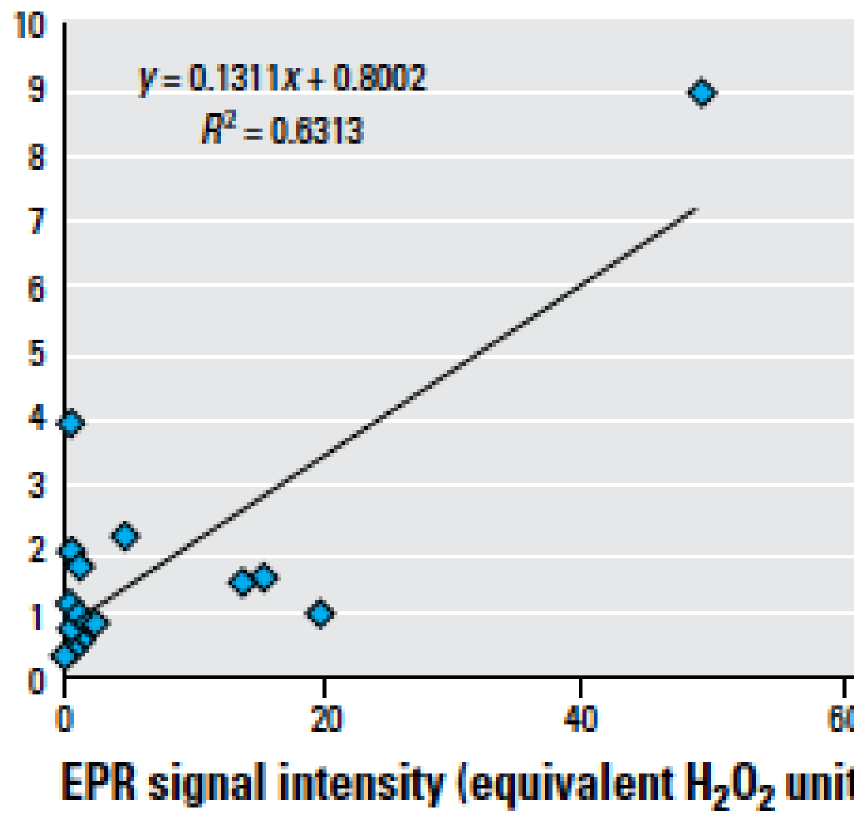
Efficacy of Simple Short-Term *in Vitro* Assays for Predicting the Potential of Metal Oxide Nanoparticles to Cause Pulmonary Inflammation

Senlin Lu,[1,2] Rodger Duffin,[1] Craig Poland,[1] Paul Daly,[1] Fiona Murphy,[1] Ellen Drost,[1] William MacNee,[1] Vicki Stone,[3] and Ken Donaldson[1]

[1]University of Edinburgh, Edinburgh, UK; [2]School of Environmental and Chemical Engineering, Shanghai University, Shanghai, China; [3]Napier University, Edinburgh, UK



re 7. DCFH fluorescence plotted against infl
enicity as measured by PMN number for
) cm²/mL surface area dose.

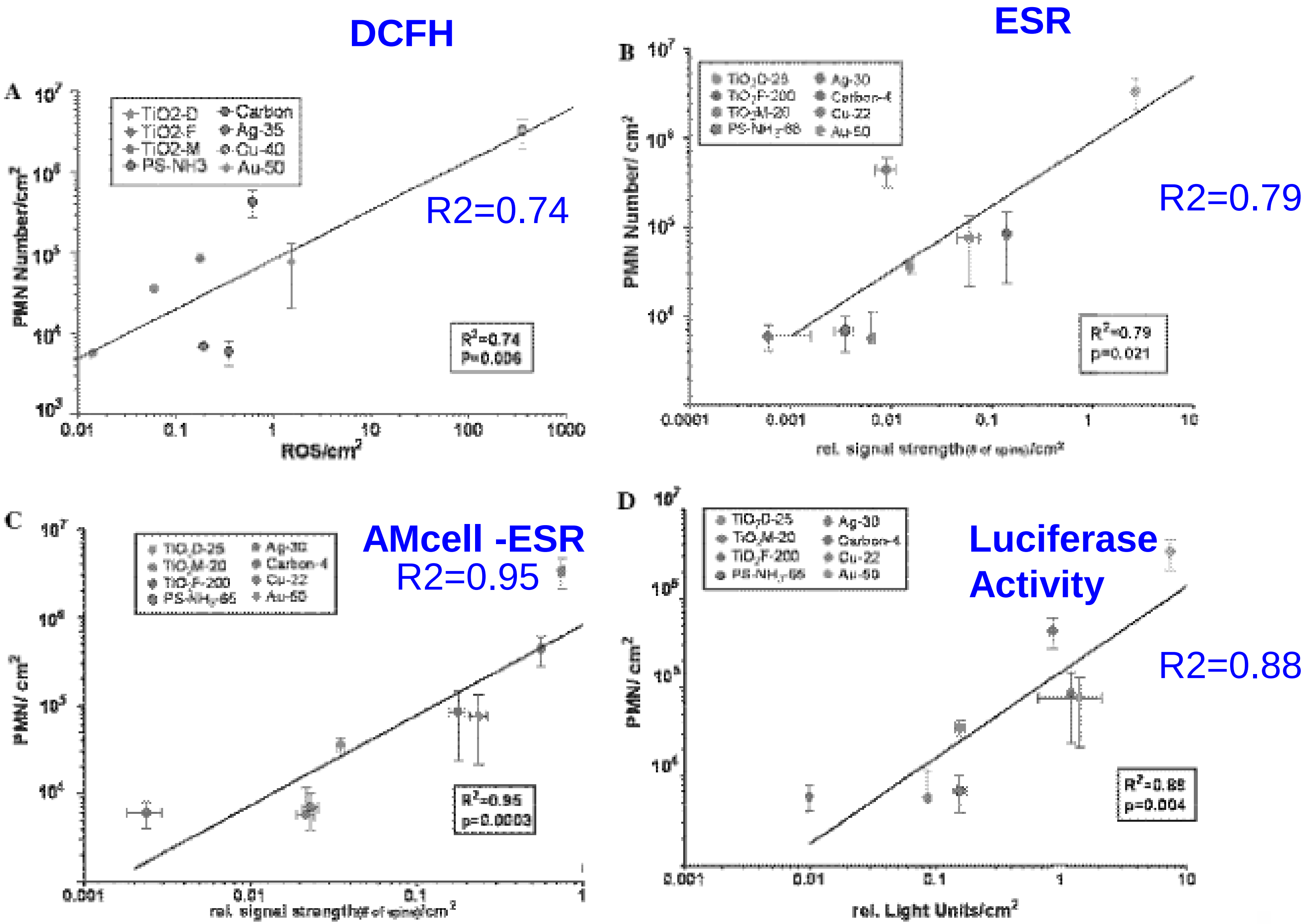


re 6. Relationship between free radical act
ability to cause inflammation *in vivo*.

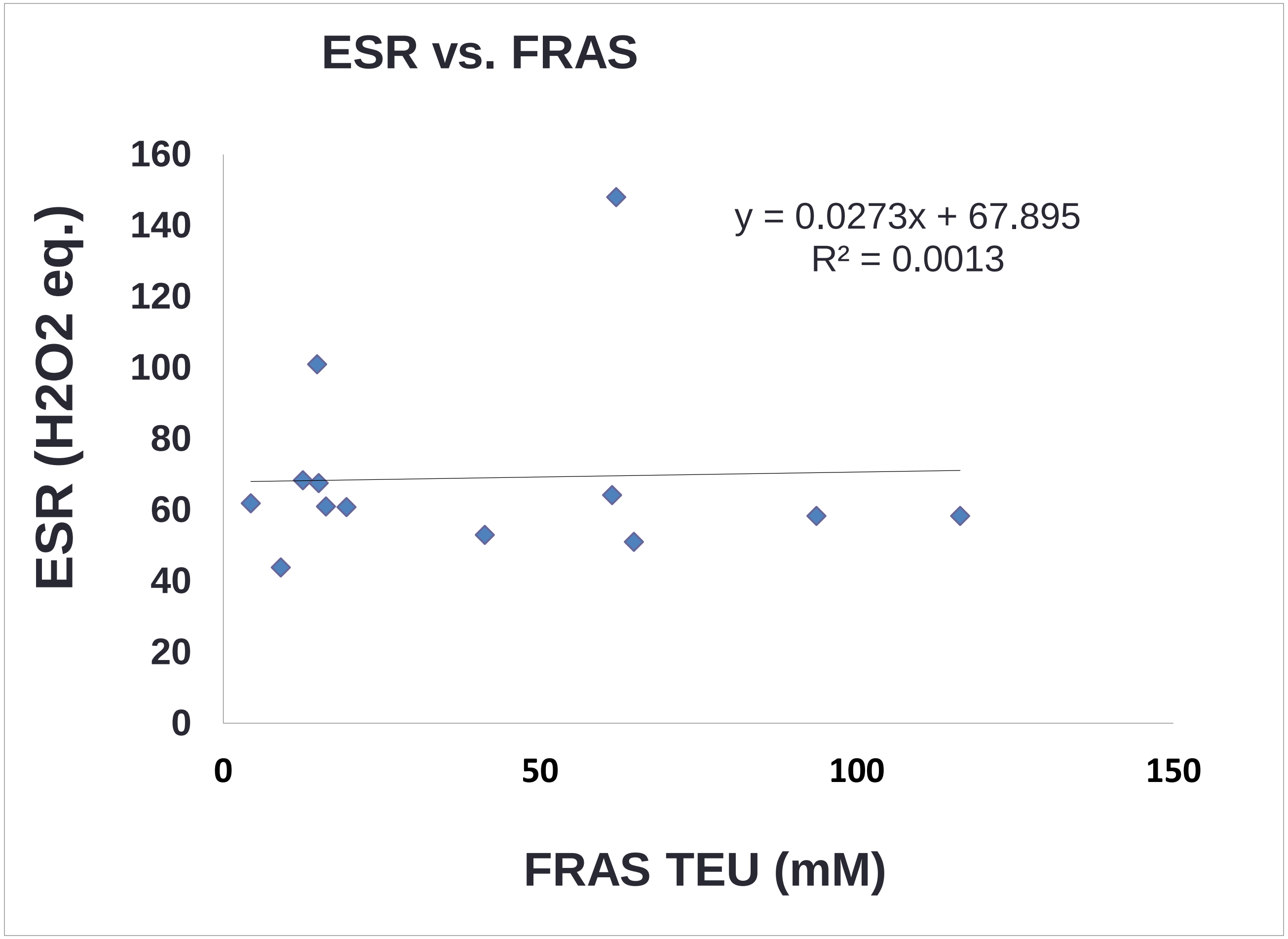
Oxidative Stress vs. Inflammation

Rushton et al 2010

P M N # / c m 2



FRAS vs ESR



Bello D, unpublished data

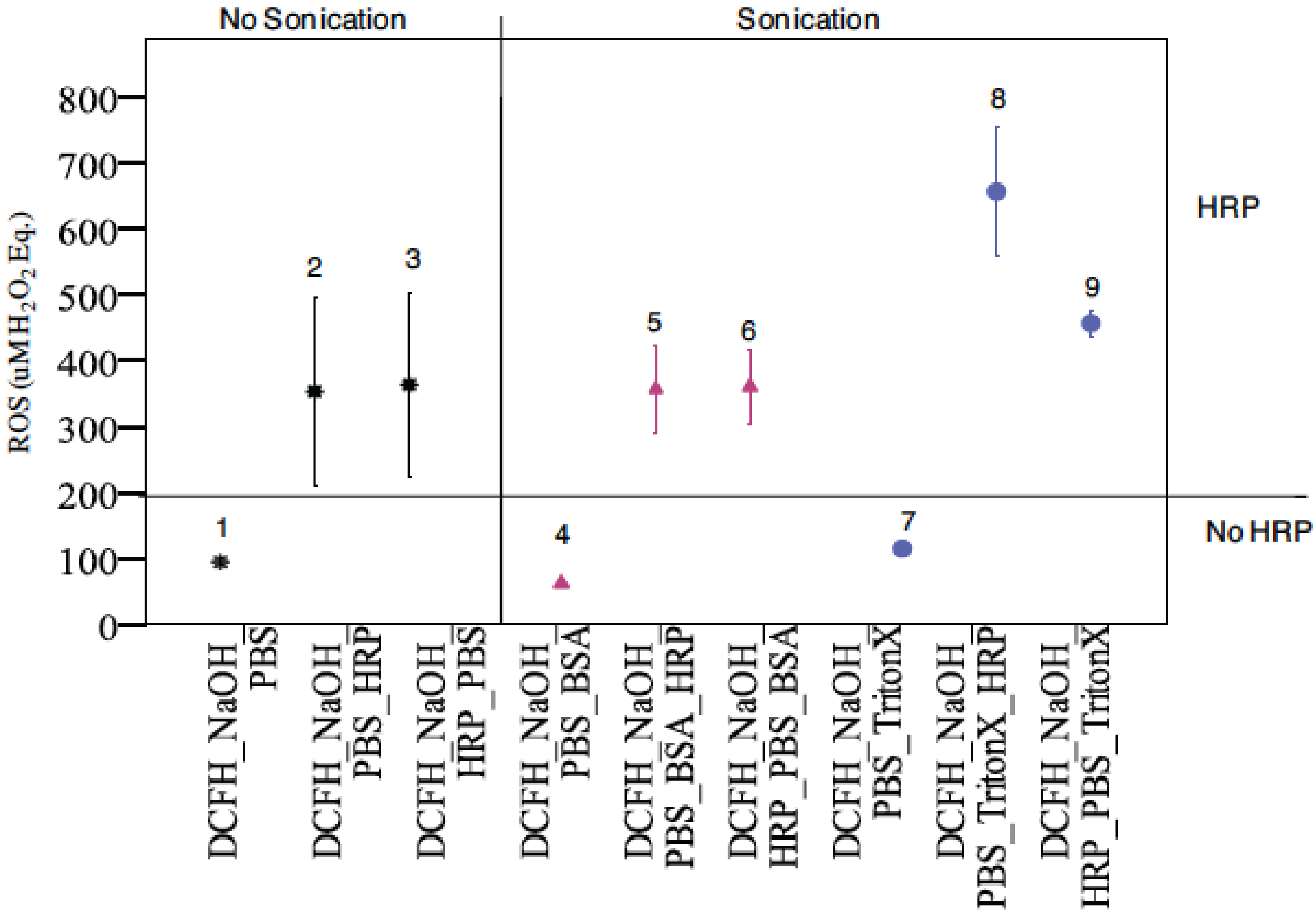
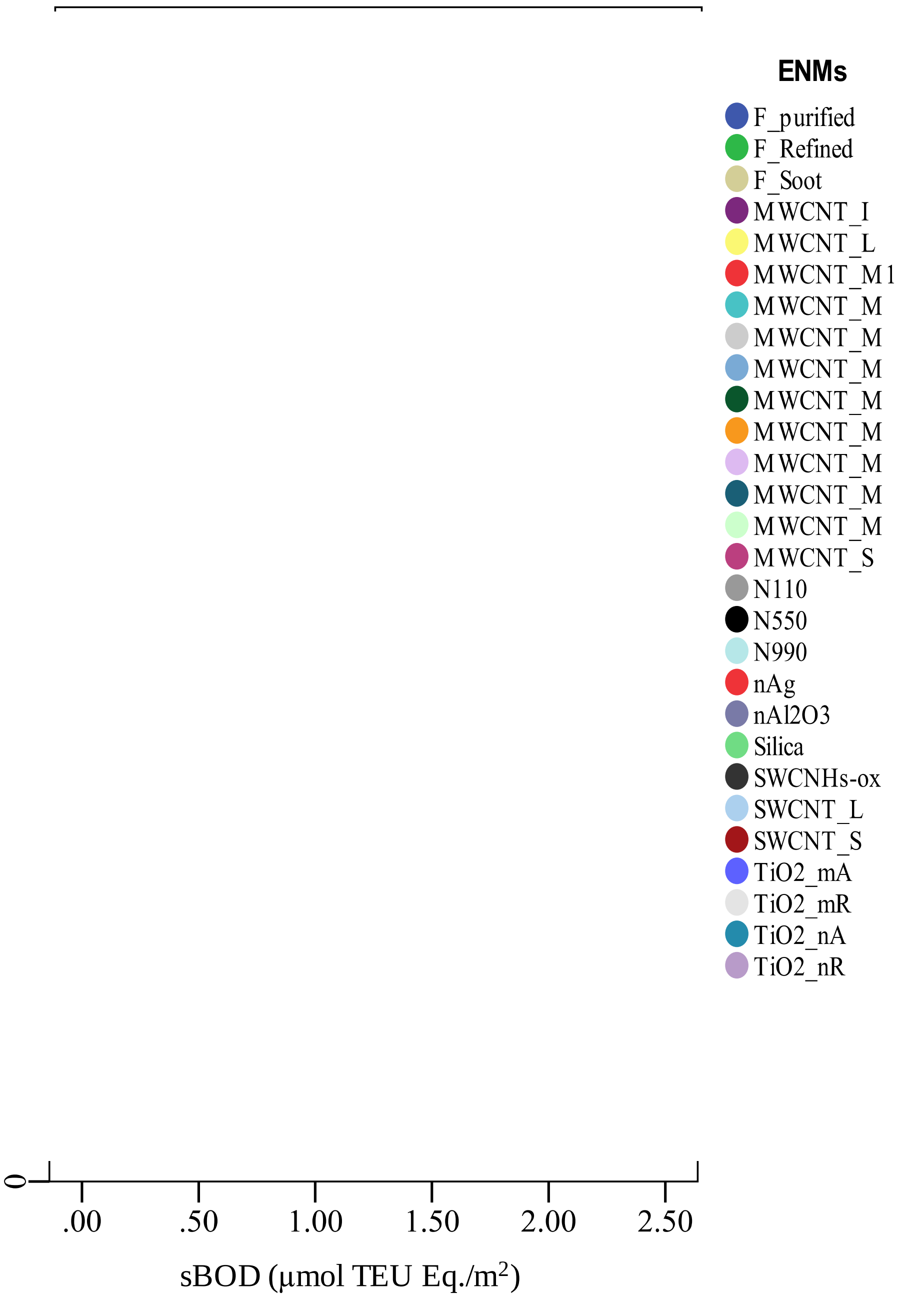


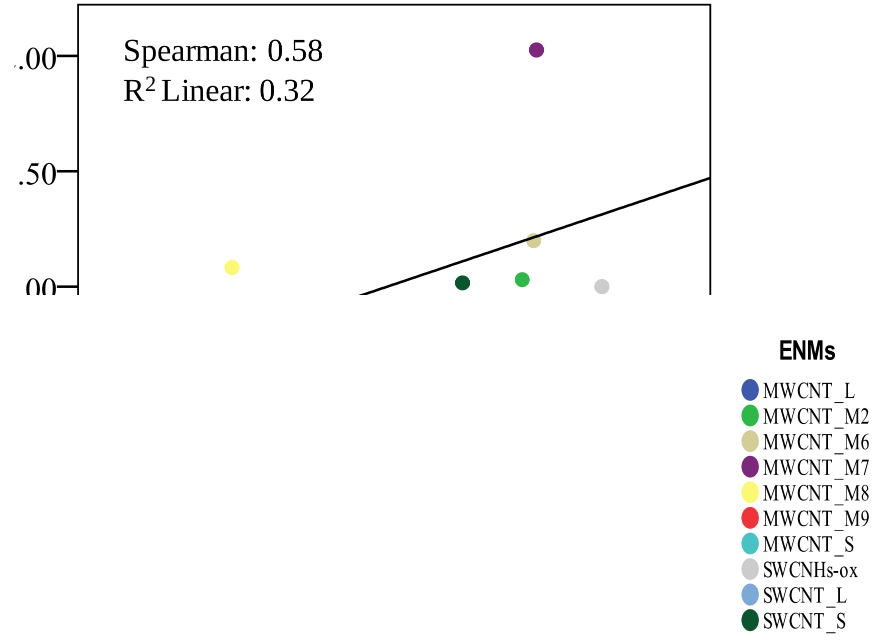
FIGURE 1. Effect 0.01U of HRP on DCFH oxidation of blanks (no nanomaterials involved) under different conditions (sequence of events and dispersion conditions). The label on the X-axis reflects actual sequence of events. HRP is undoubtedly involved in DCFH oxidation and the magnitude of the effect spans approximately an order of magnitude compared to blanks without HRP, depending on the experimental conditions.

Pal et al 2011 Dose-Response

(A)


ENMs
F_purified
F_Refined
F_Soot
MWCNT_I
MWCNT_L
MWCNT_M1
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_M
MWCNT_S
N110
N550
N990
nAg
nAl2O3
Silica
SWCNHs-ox
SWCNT_L
SWCNT_S
TiO2_mA
TiO2_mR
TiO2_nA
TiO2_nR
0
.00
.50
1.00
1.50
2.00
2.50
sBOD (μmol TEU Eq./m²)

(C)


Spearman: 0.58
R² Linear: 0.32
.00
.50
ENMs
MWCNT_L
MWCNT_M2
MWCNT_M6
MWCNT_M7
MWCNT_M8
MWCNT_M9
MWCNT_S
SWCNHs-ox
SWCNT_L
SWCNT_S

Comparison of FRAS & DCFH for the 28 ENM

N=28

		FRAS	
		Positive (21/28)	Negative (7/28)
DCFH	Positive (10/28)	10	0
	Negative (18/28)	11	7

Pal et al 2013 J Nano Research

Table 5 Comparison of intracellular oxidative stress elicited by ENMs (GSH assay) with acellular oxidative stress (via DCFH and FRAS)

Nanomaterials Evaluated	Acellular assay responses		Cellular response
	DCFH	FRAS	
CB N-550	–	+	+
SWCNT_S	++	++	++
MWCNT _M1	–	+	+
MWCNT_I	–	+	++
SWCNHs-ox	+	++	+++
TiO_2_*n*A	–	+	++
Silica	–	–	_

(–) Indicates a response below the blank value (a negative response)

(+) Indicates a slightly positive ENM response relative to the overall response scale (between 10 and 50 percentile)

(++) Indicates a moderately positive ENM response relative to the overall response scale (between 50 and 75 percentile)

(+++) Indicates highly positive ENM response relative to the overall response scale (between 75 and 95 percentile)

Acellular vs. Intracellular GSH/GSSG

Pal et al 2014
J Nano Research

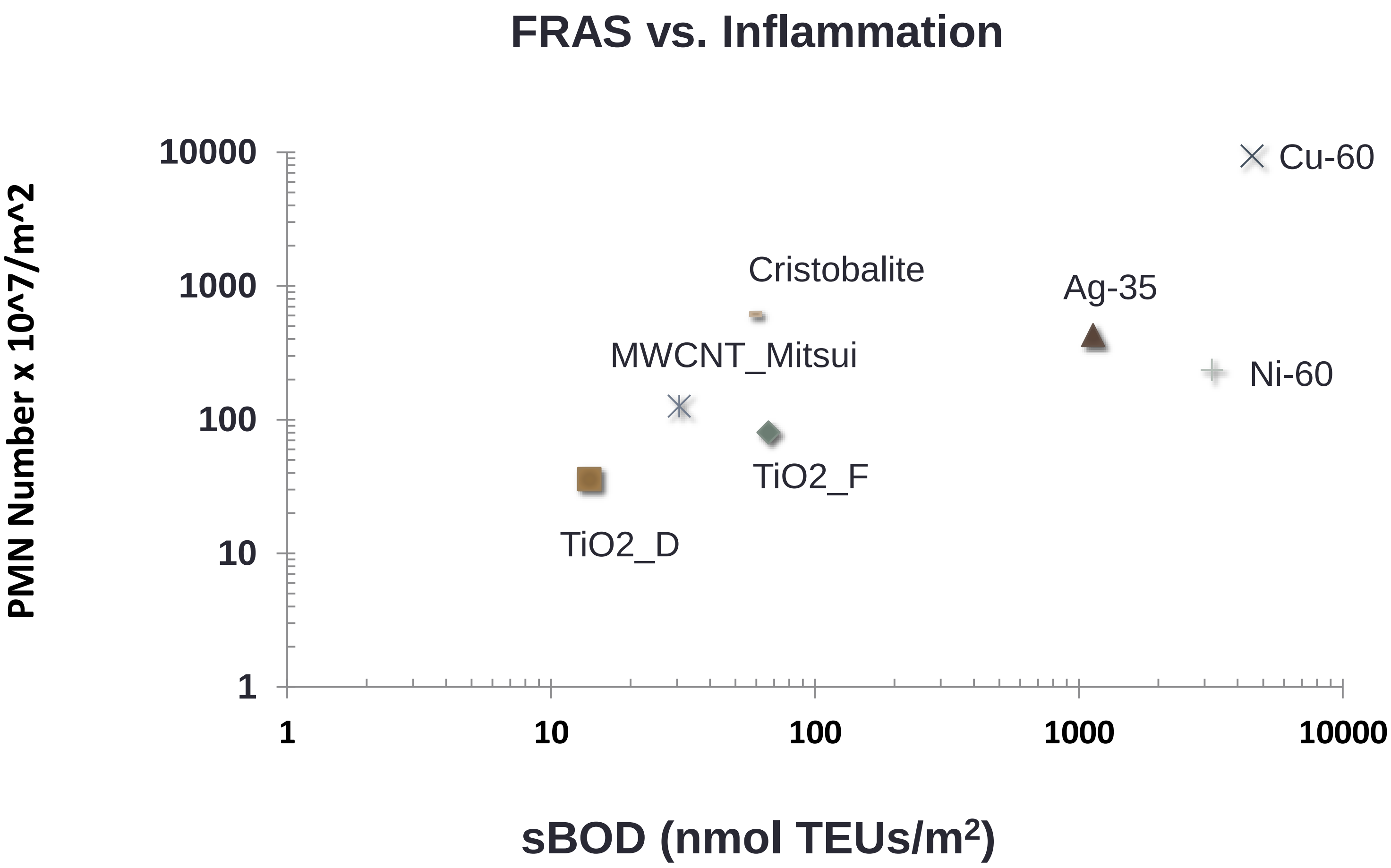
Table 2: Relationship between FRAS and DCFH responses and transition metal content in tested ENMs examined via Spearman Correlation Coefficients (r values) and Partial Correlation (SSA controlled).

ENMs Set	Assay	Transition metal						
		Fe	Cr	Co	Mo	Mn	Zn	Ni
28 ENMs (Spearman)	FRAS	0.57**	0.67**	0.67*	0.59**	0.53**	-----	0.51**
	DCFH	0.50*	0.67**	0.67**	0.57**	0.63**	-----	0.54**
28 ENMs (Partial)	FRAS	-----	0.48*	0.67**	-----	-----	-----	-----
	DCFH	-----	-----	0.36*	-----	-----	-----	-----

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

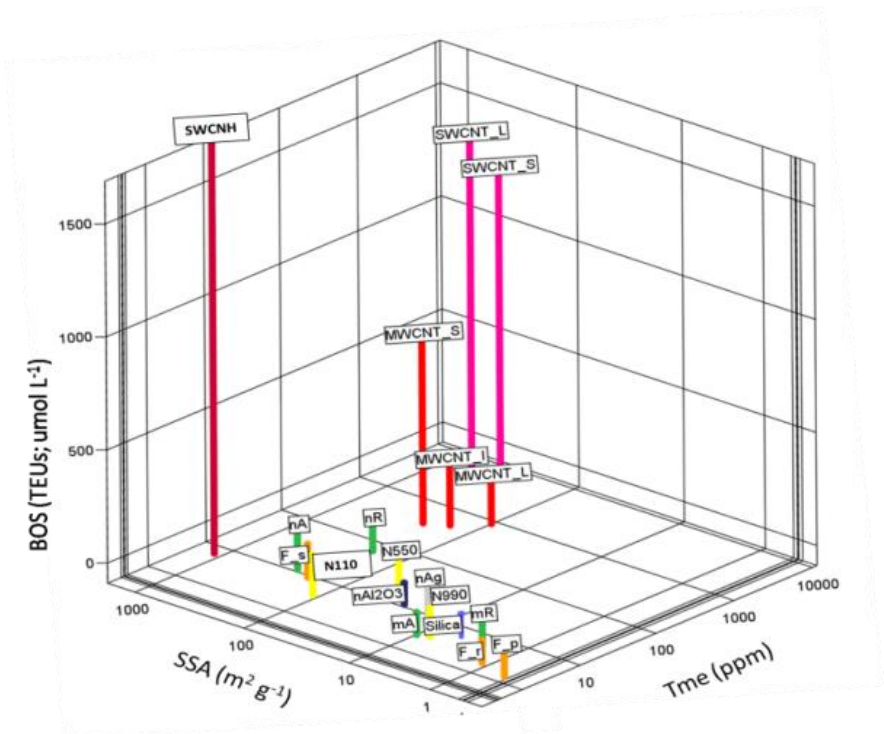
Pal et al J Nano Research 2013

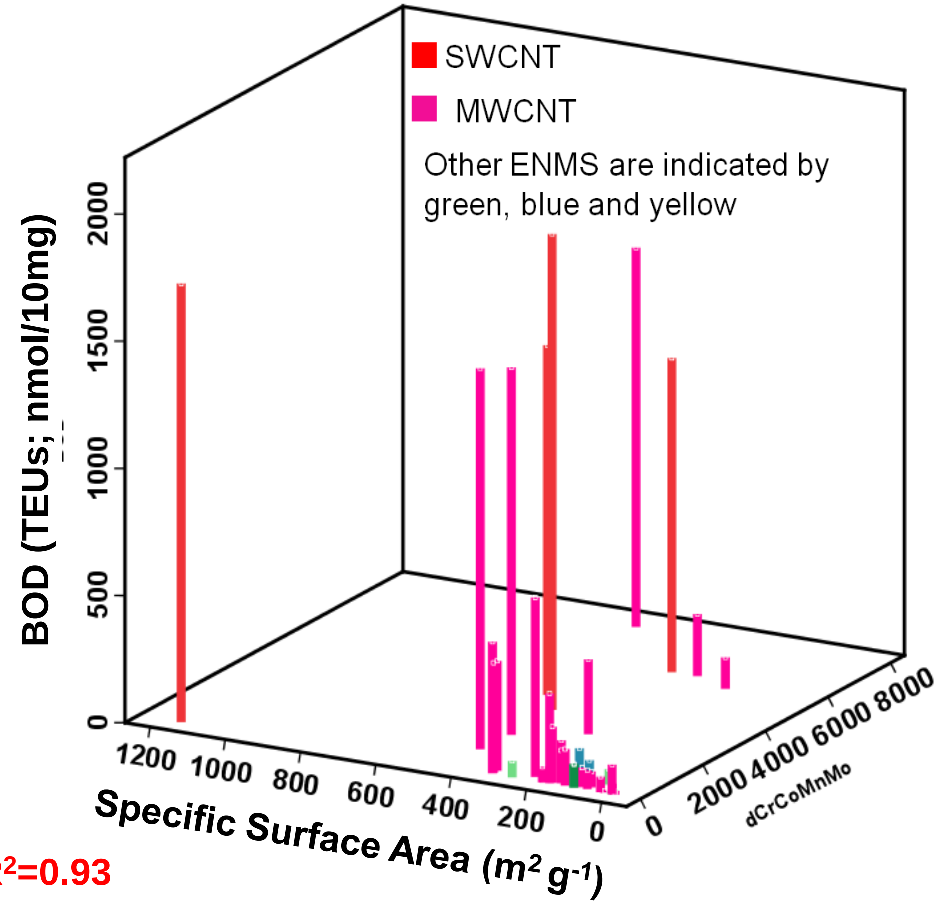
Oxidative Stress vs. Inflammation



Bello Unpublished data; PMN data: Alison Elder, U. Rochester

Physicochemical Properties & FRAS Oxidative Stress





BOD (TEUs) = 0.75 * SSA (m^2g^{-1}) + 0.418*T_{Me} (ppm); R^2=0.93

Bello, Hsieh et al 2010 Nanotoxicology

BOD ~ with SSA and the total content of Redox-active metals

Hsieh, Bello et al 2013

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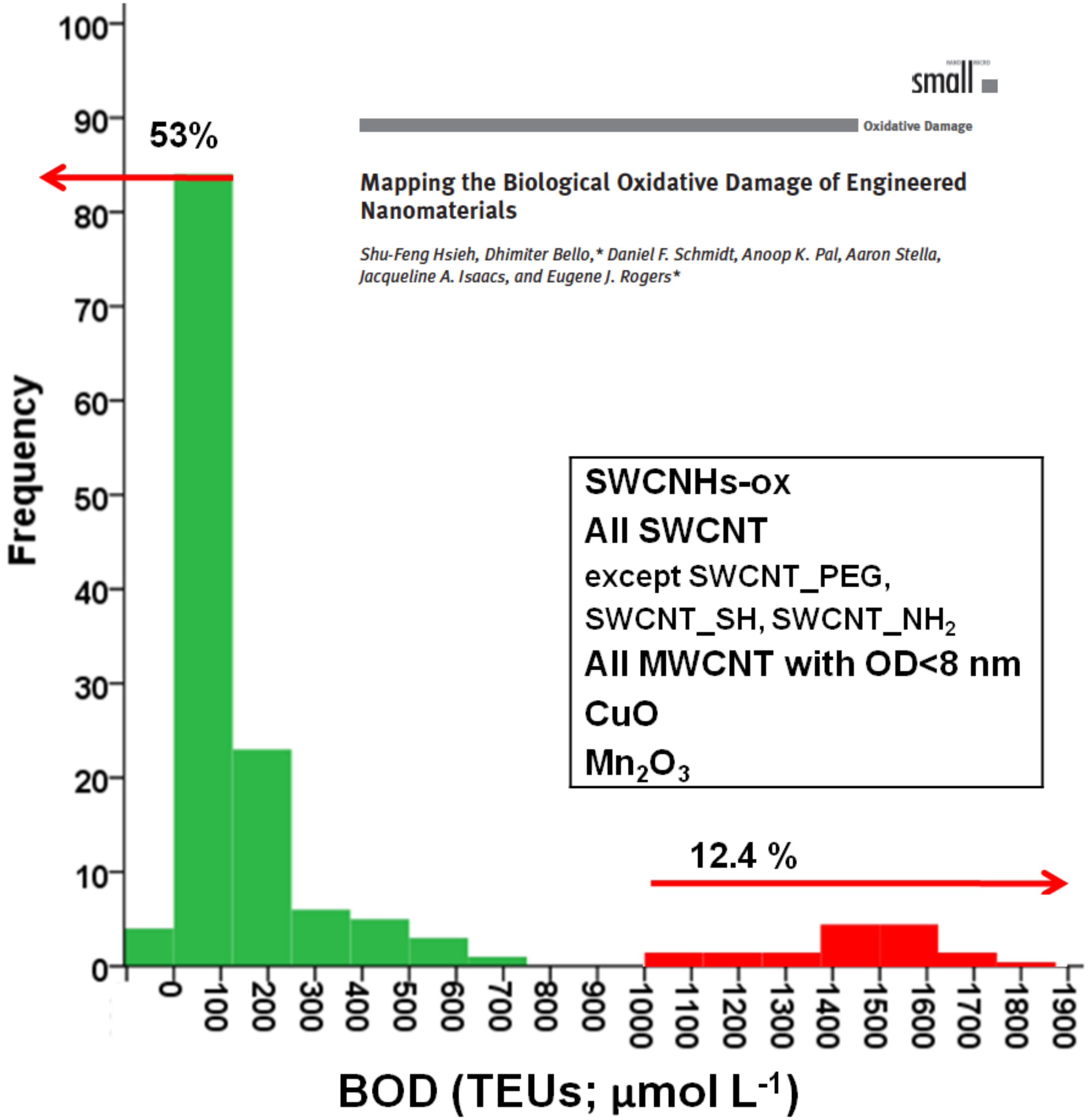
Distribution of FRAS BOD Values of 145 Tested ENMs



1. BOD is expressed as Trolox equivalent units. (TEUs , µmol L^{-1}).
2. Total antioxidant capacity of normal blood serum is 535 ±15 µmol L^{-1} TEUs.
3. 1000 TEUs BOD, near complete depletion of the antioxidants pool (5mg/mL).
4. 15 TEUs = The non-significant BOD
5. 25% of ENM <15 TEUs

Hsieh et al 2013 Small

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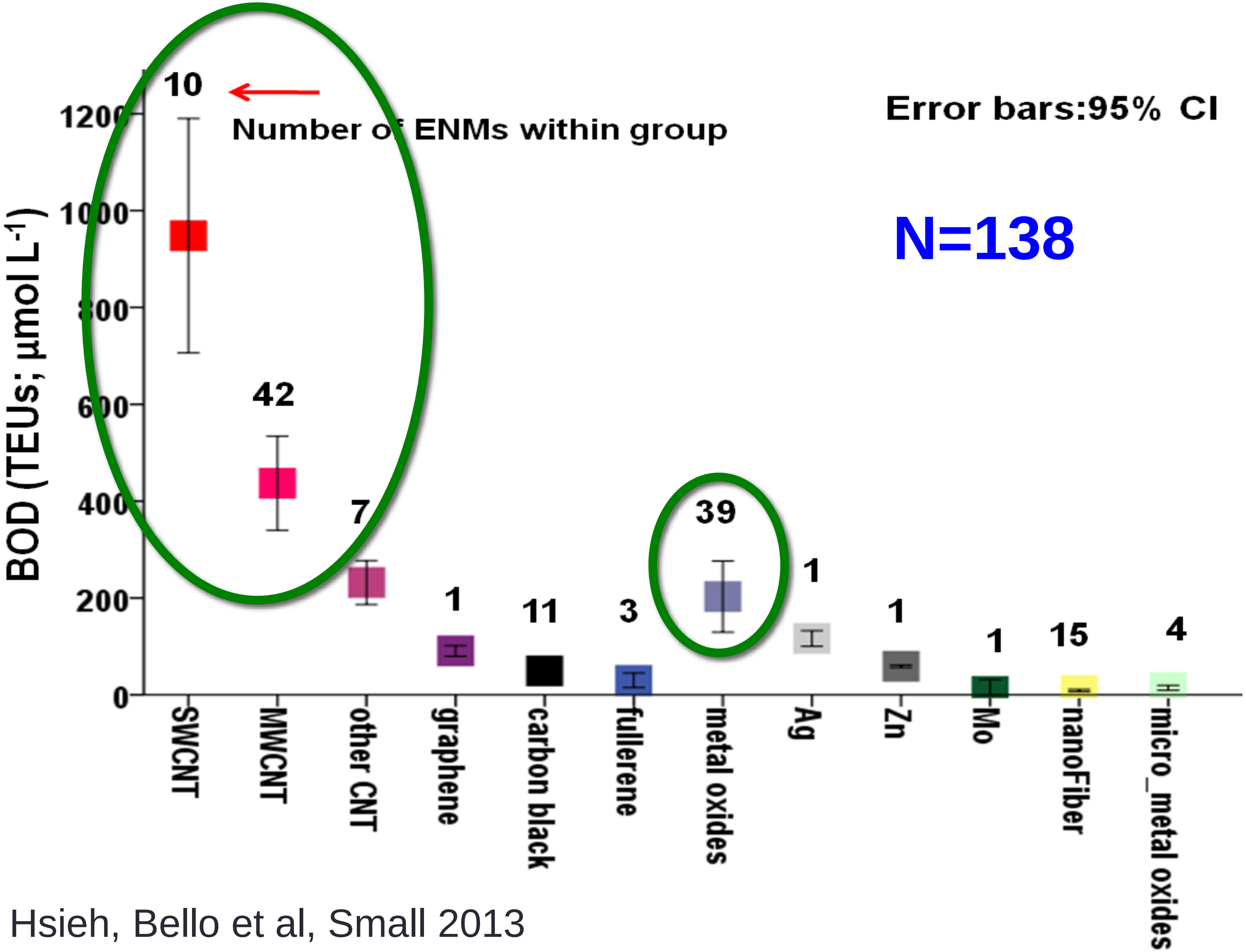
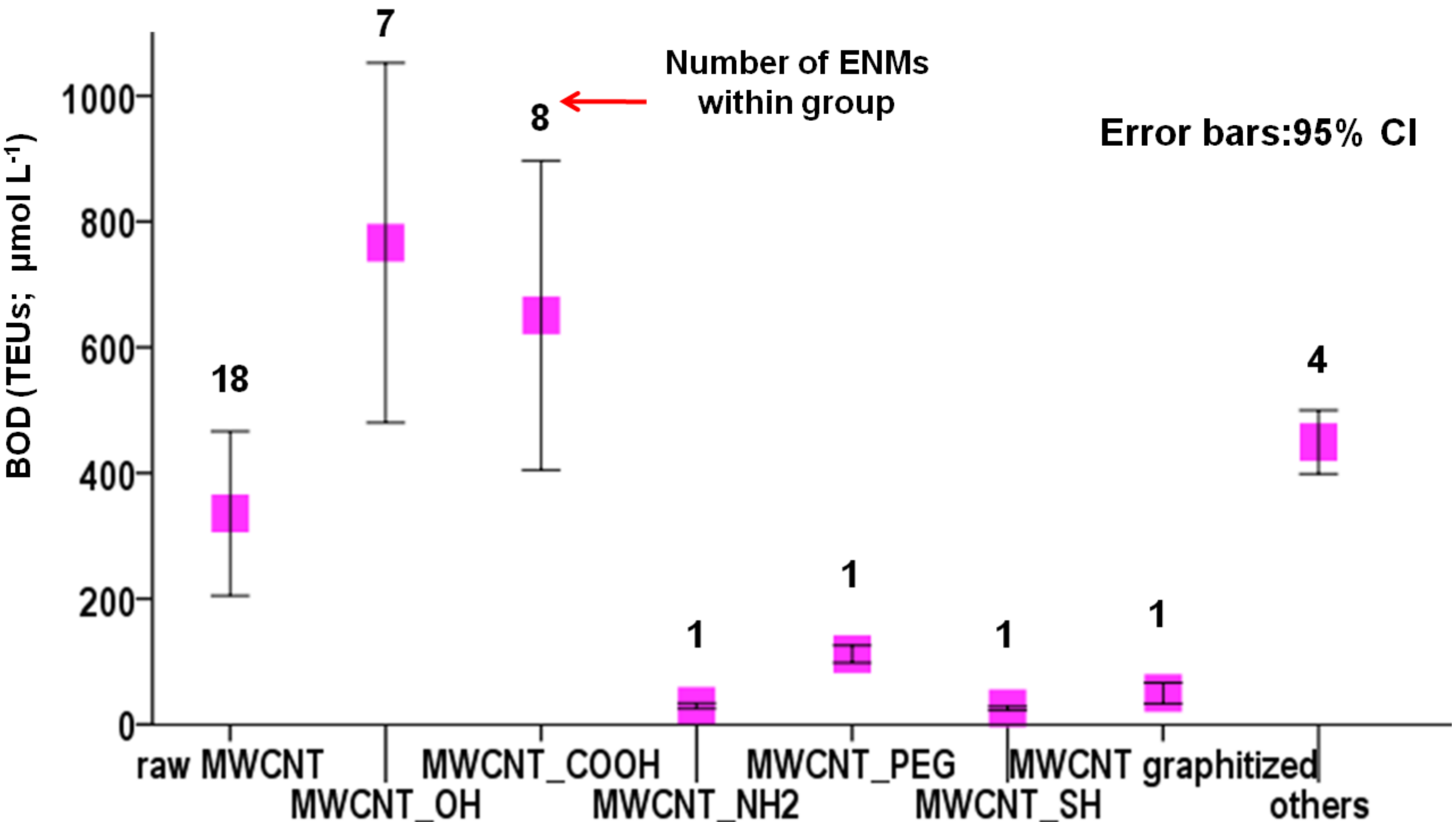
Between-Class Variations in BOD



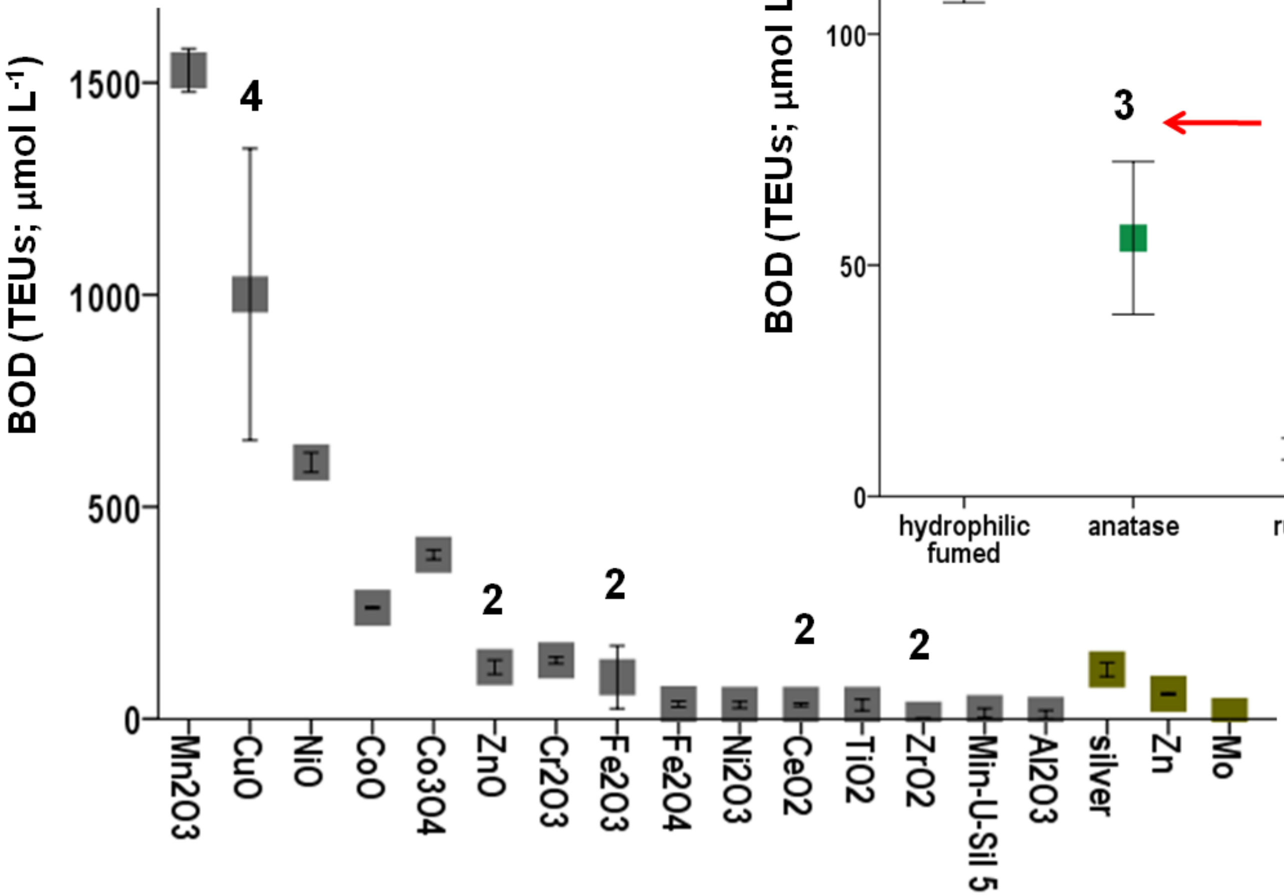
Hsieh, Bello et al, Small 2013

Within-Class Variation in BOD, CNTs

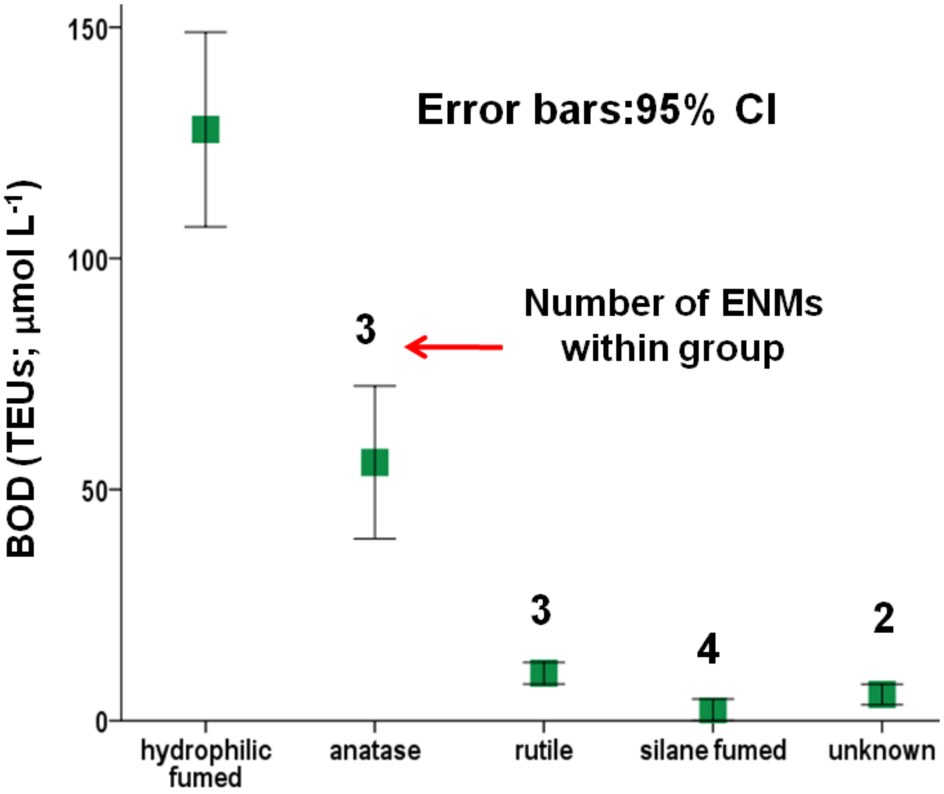
Hsieh, Bello et al Small 2013



Metal Oxides

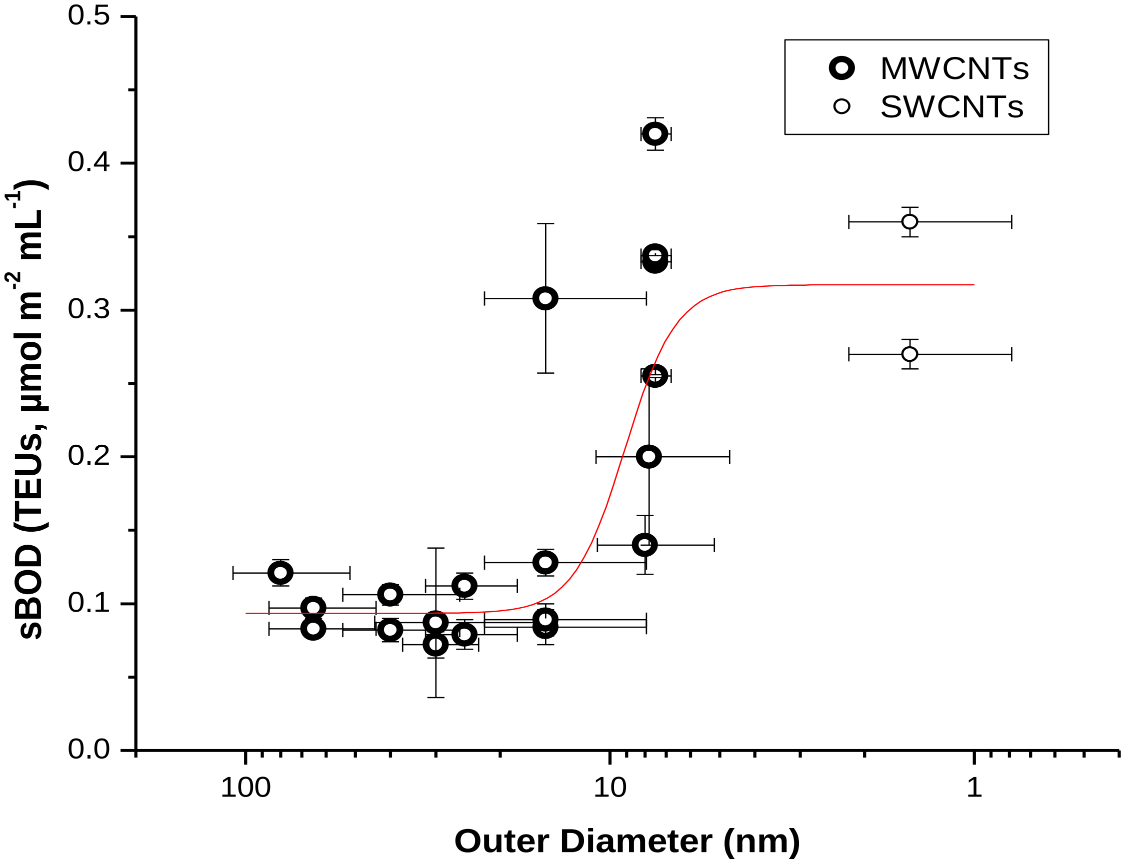


TiO$_2$



Hsieh, Bello et al Small 2013

Effect of CNT OD size on BOD

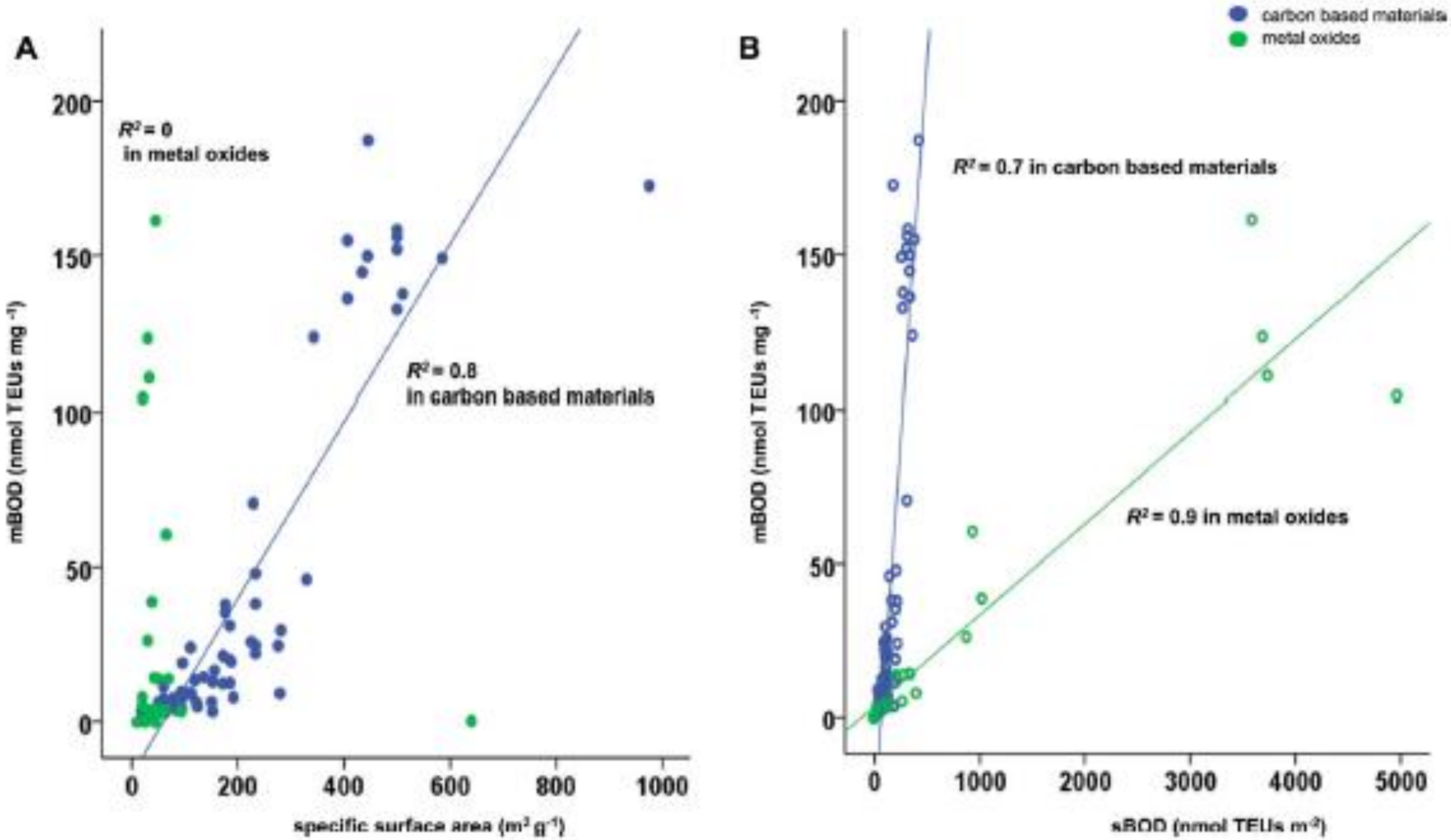


Surface Activity of narrow CNTs is much higher than for larger CNTs

Hsieh, Bello et al 2011 Nanotoxicology

Center for High-rate Nanomanufacturing

Surface Activity vs. ENM Class



Hsieh, Bello et al Small 2013

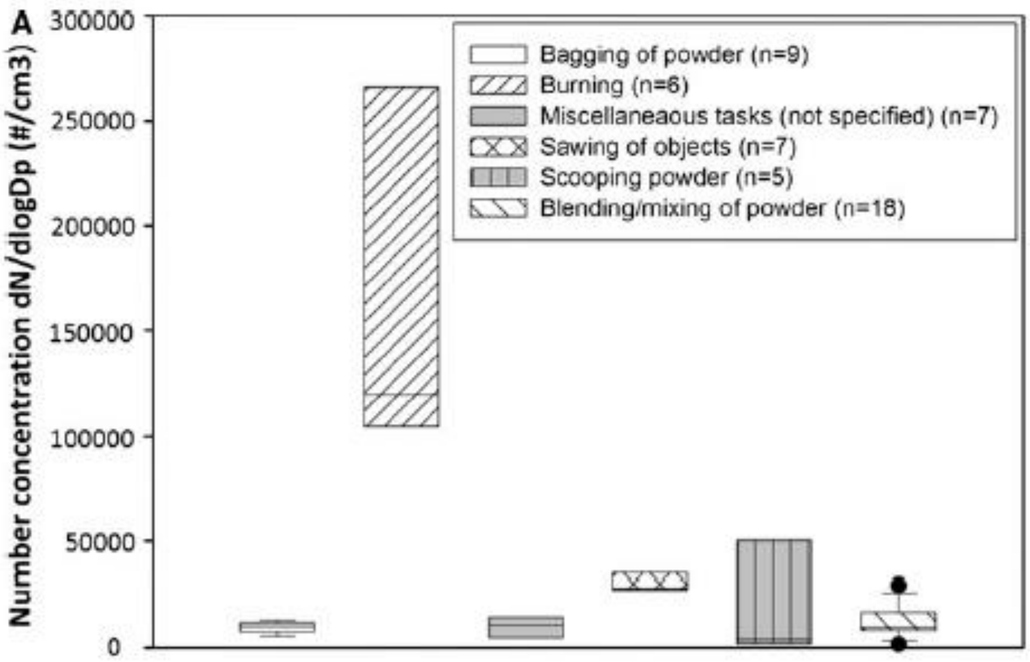
Outline

- Surface in Inhalation Dosimetry
  - Surface Area & Activity
  - Bench top Technologies and Options
- Surface in ENM Exposure Assessment
  - Gaps & Needs
- Near Real-time monitoring of SAr and SAc

Table 3 Range of arithmetic means of particle number concentration (#/cm^3) of particles <100 nm and surface area concentration (μm^2/cm^3) during nano- and non-nano-activities for the various subcategories

Activity	Range of arithmetic means during activity	
	Number conc. by SMPS (particles/cm^3)	Surface area concentration by LQ1-DC (μm^2/cm^3)
Production—commercial scale		
Nano-activity (=A)	1,661–39,087 (n = 12)	25–74 (n = 8)
No activity (=B)	1,339–23,566 (n = 8)	21–69 (n = 8)
Production—non-commercial scale		
Nano-activity (=A)	3,887–21,441 (n = 7)	43–129 (n = 6)
No activity (=B)	2,040–12,919 (n = 7)	35–93 (n = 3)
Down-stream use—commercial scale		
Nano-activity (=A)	6,272–7,8376 (n = 9)	17–88 (n = 11)
No activity (=B)	6,242–32,515 (n = 7)	18–51 (n = 10)
Down-stream use—non-commercial scale		
Nano-activity (=A)	234–380,494 (n = 57)	36–173 (n = 13)
No activity (=B)	199–34,507 (n = 55)	27–147 (n = 10)

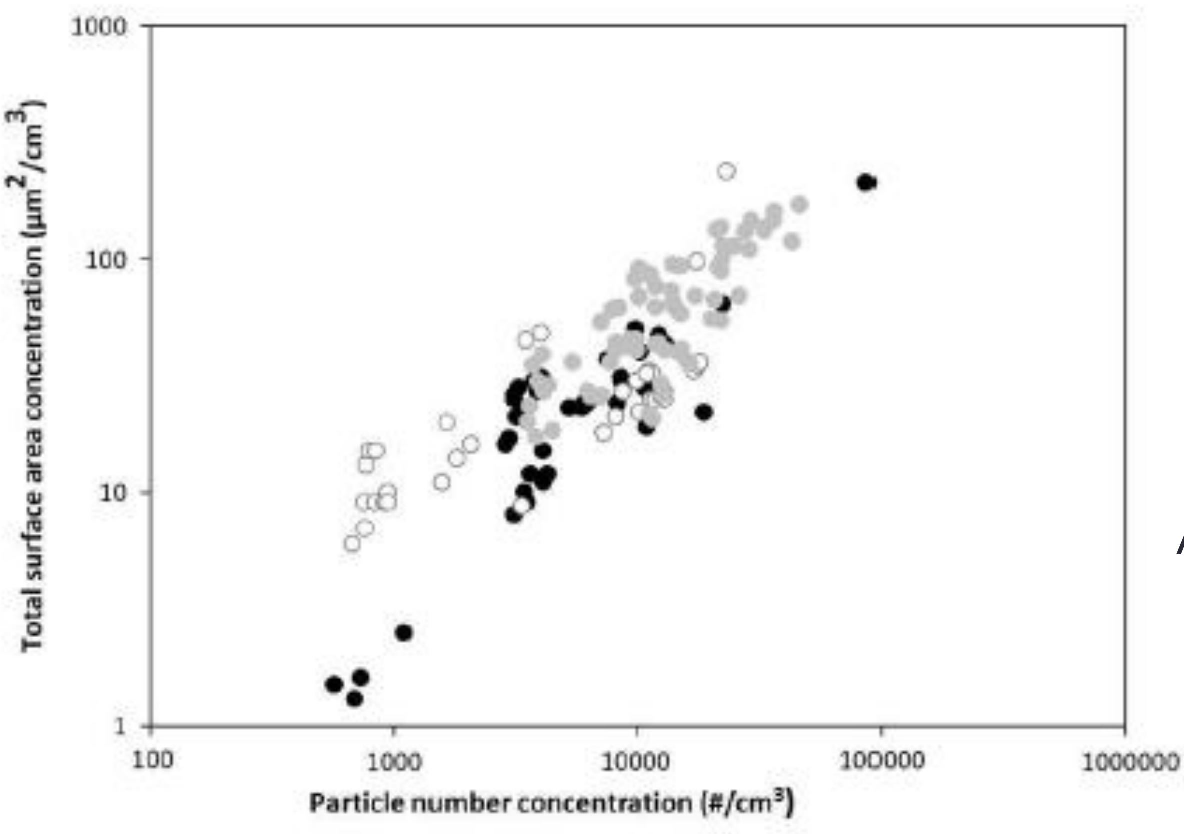
Exposures to ENM by Task



Brouwer et al J Nanoparticle Res 2013, 15, 2090

Surface Area vs. Number Concentration

NSAM



SMPS

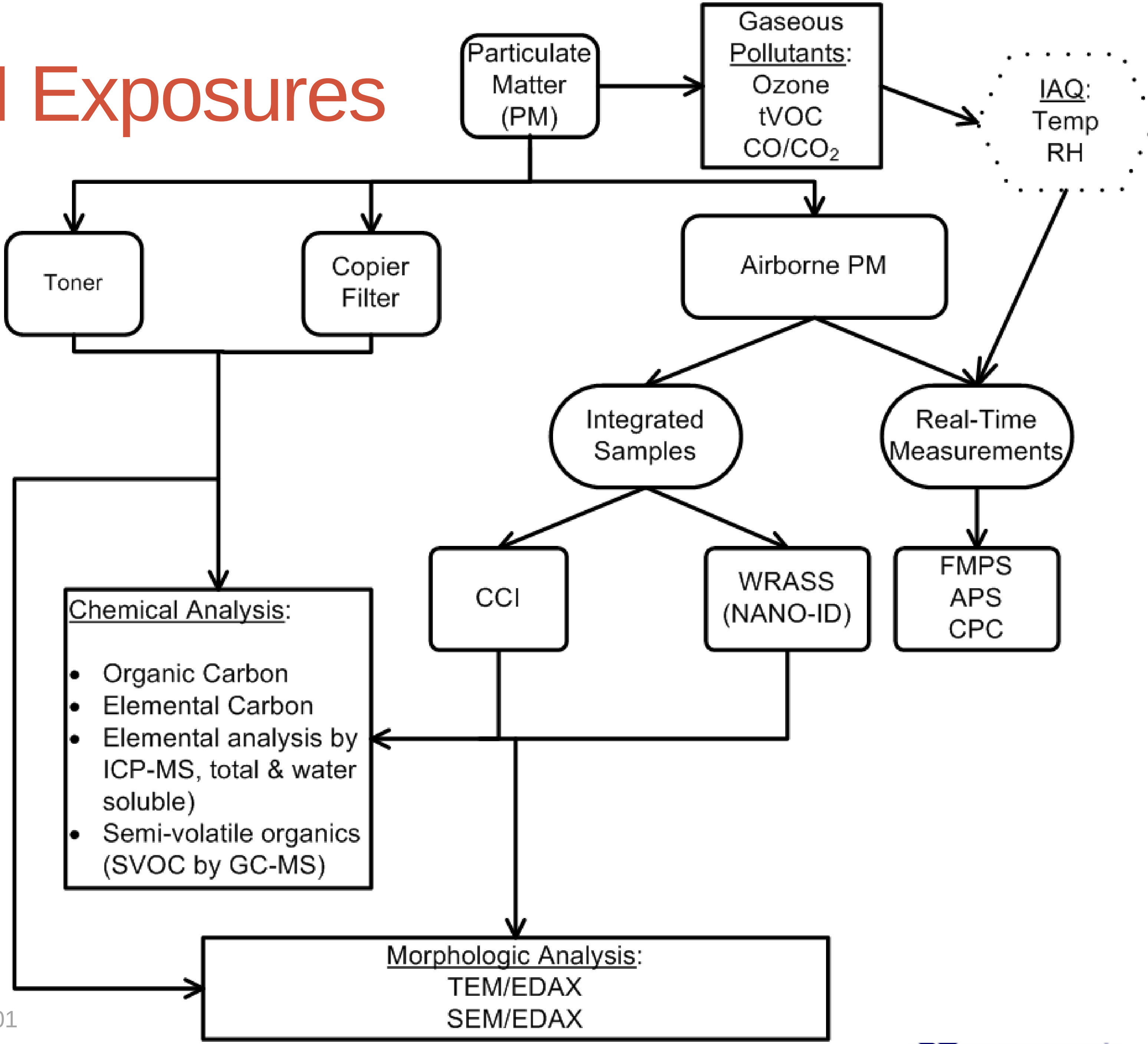
<100 nm & ALVEOLAR

>100 nm & TB

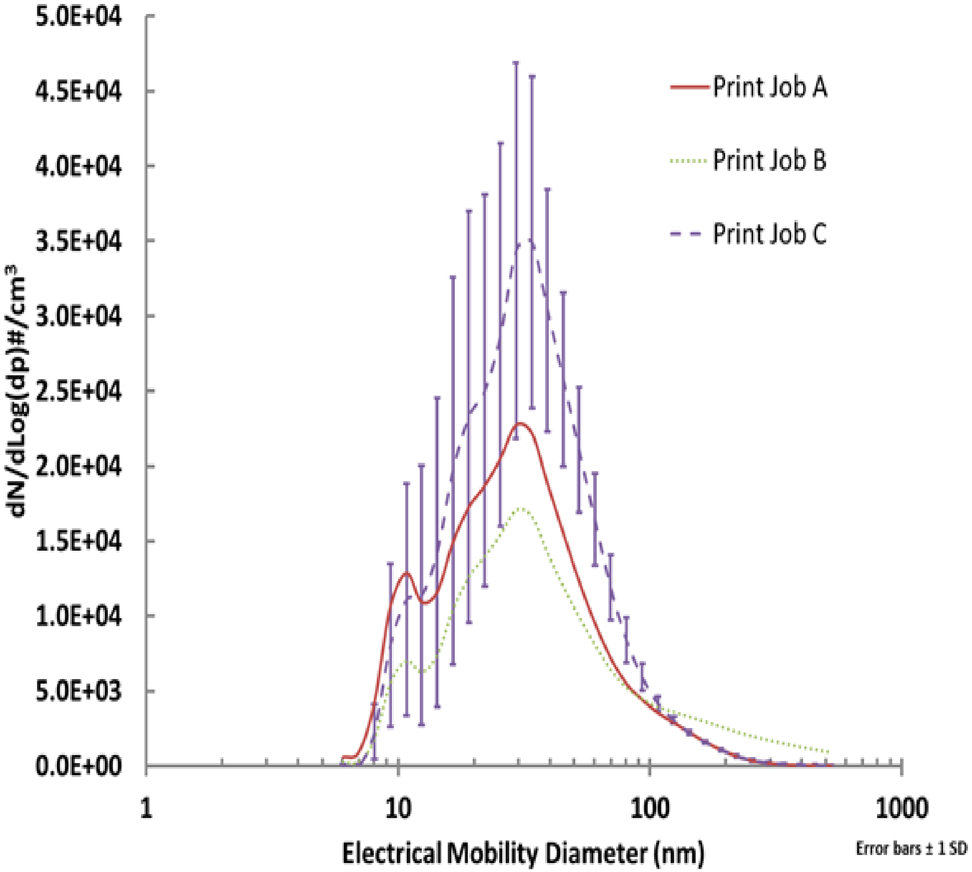
200-1000 nm & ACTIVE SA (LQ1-DC)

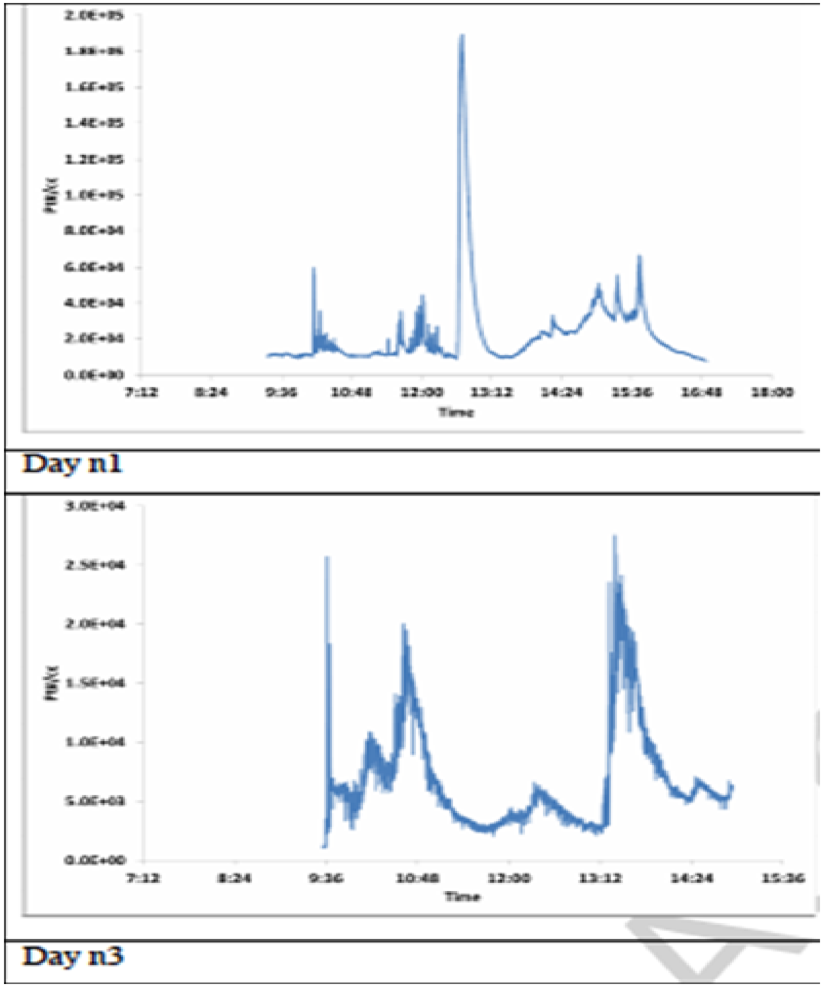
Brouwer et al Journal of Nanoparticle Research, 2013 15(11); DOI:10.1007/s11051-013-2090-7

ENM Exposures



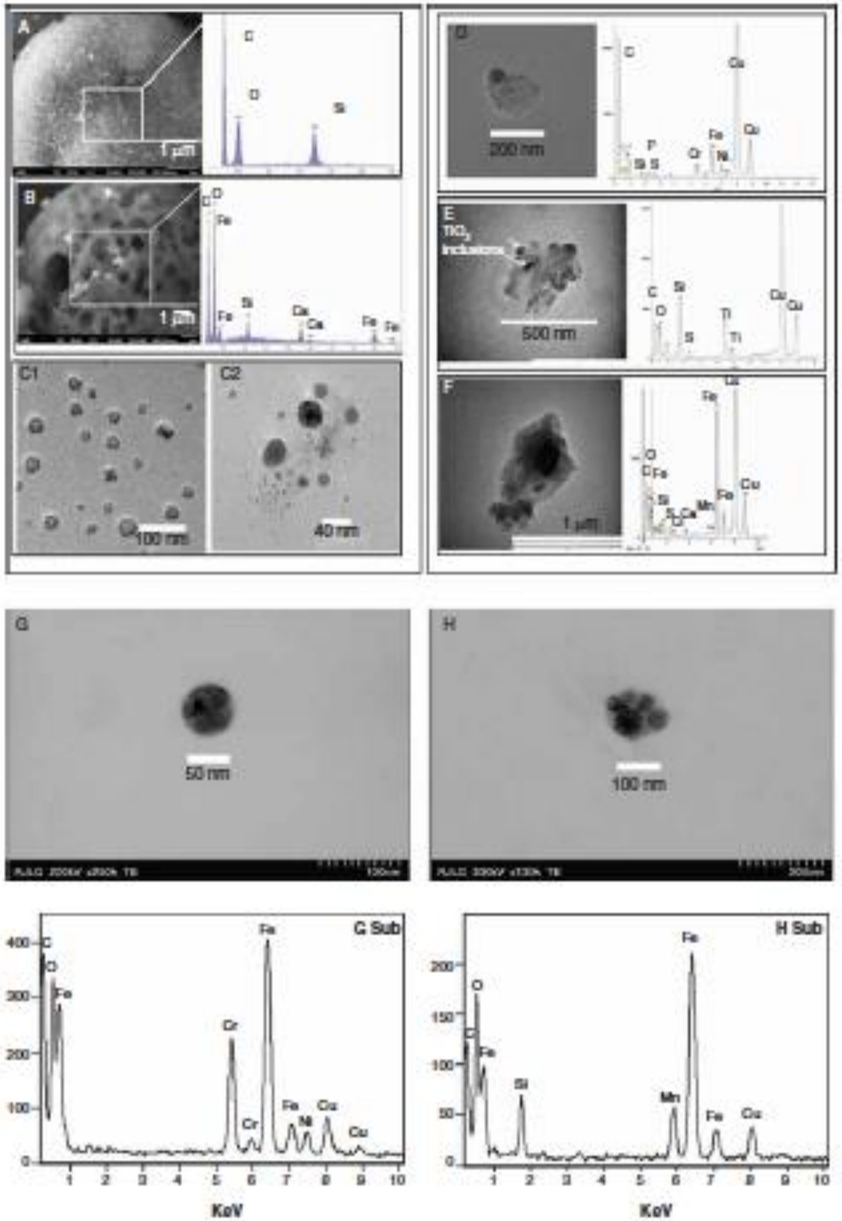
Nanoparticle Emissions from Commercial Photocopiers

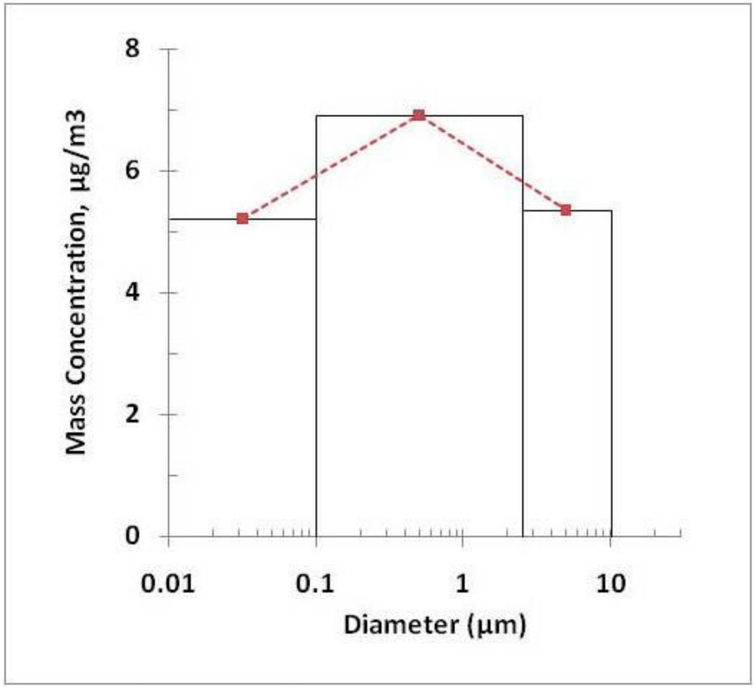




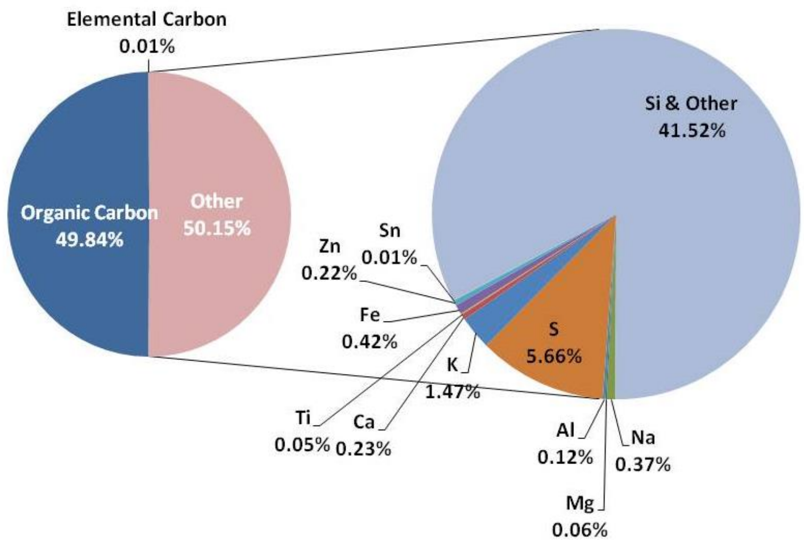
Bello et al Nanotoxicology 2012

Morphology & Chemistry





Ultrafine (PM <0.1 µm), 5.21 ug/m³
Elements, 0.47 ug/m³



Bello et al Nanotoxicology 2012

Important Real-World Lessons

- NP Exposures are often to MIXTURES
- PCM Properties along the life cycle of NEPs may be DIFFERENT from input ENMs
  - Toxicological properties – likely DIFFERENT (by how much & what direction?)
- Multi-metric approach – necessary
  - Sufficient ?
  - Interpretation ?
- Exposure- dose equivalency for in vitro or in vivo work…
  - mass, number, surface area, elemental composition, ?,

Outline

- Current Metrology & Exposure Monitoring for ENM
- Surface Area
- Surface Activity – what does it tell us?
  - Benchtop Technologies and Options
  - Validity of the Concept
- Near Real-time monitoring of SAr and SAc

Nanodevices (FP7 project)

http://www.nano-device.eu/index.php?id=328

Devices

No.	Name of Device	WP	Measured physical metric	Type of nanoparticles	Particle size range	Ability to seperate ENP from background
1	Low-cost total active surface area monitor	6	Total active surface area	Any	10 nm - 3 µm	No
2	NanoGuard	7	Number concentration, size distribution, morphology finger print	Any	<20 nm - 450 nm	to be determined
3	Real-Time CNT Monitor	8	Number concentration	CNTs	all CNT sources	Yes
4	Personal Nano-sampler	8	Mass concentration and size distribution of target ENP	Any	2 nm - 5 µm	No, only by further offline chemical analysis
5	Sampler/Preseparator for aerosol fraction deposited in the anterior nasal region	8	depending on the metrics of the used monitor	Compact isometric particles and agglomerates	5 nm - 400 nm	No, only by further offline chemical analysis
6	Sampler/Preseparator for aerosol fraction deposited in the gas exchange region	8	depending on the metrics of the used monitor	Compact isometric particles and agglomerates	20 nm - 5 µm	No, only by further offline chemical analysis
7	NanoDevice	9	Particle number and size	Any (no corrosive particles)	10 nm - 27 µm	Yes
8	MEMS-based airborne nanoparticle sensor	10	Mass concentration & chemical composition	Any, but no CNTs	5 nm - 300 nm	Yes
9	Catalytic Activity Aerosol Monitor (CAAM)	11	Catalytic activity concentration	Any with catalytic activity	No limitation in principle	Yes
10	CNT-detect	12	mass concentration	CNTs	all CNT sources	Yes

(Click for more information)

1. Low-cost total active surface area monitor
2. NanoGuard
3. Real-Time CNT Monitor
4. Personal Nano-sampler
5. Sampler/Preseparator for aerosol fraction deposited in the anterior nasal region
6. Sampler/Preseparator for aerosol fraction deposited in the gas exchange region
7. NanoDevice
8. MEMS-based airborne nanoparticle sensor
9. Catalytic Activity Aerosol Monitor (CAAM)
10. CNT-detect

Near-Real Time ROS Is Almost Here

Development and testing of an online method to measure ambient fine particulate Reactive Oxygen Species (ROS) based on the 2′,7′-dichlorofluorescin (DCFH) assay

L. E. King and R. J. Weber

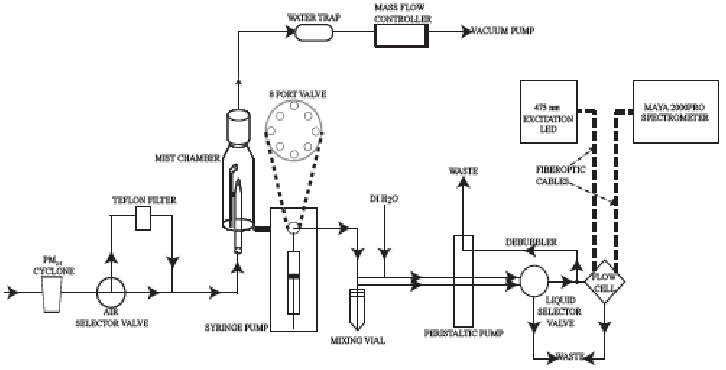
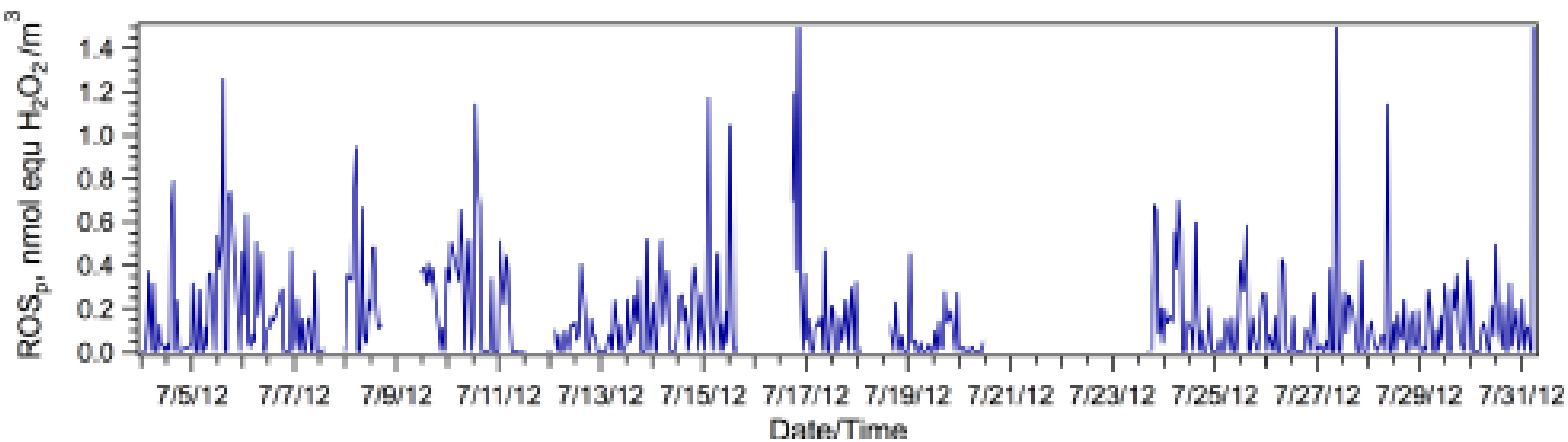
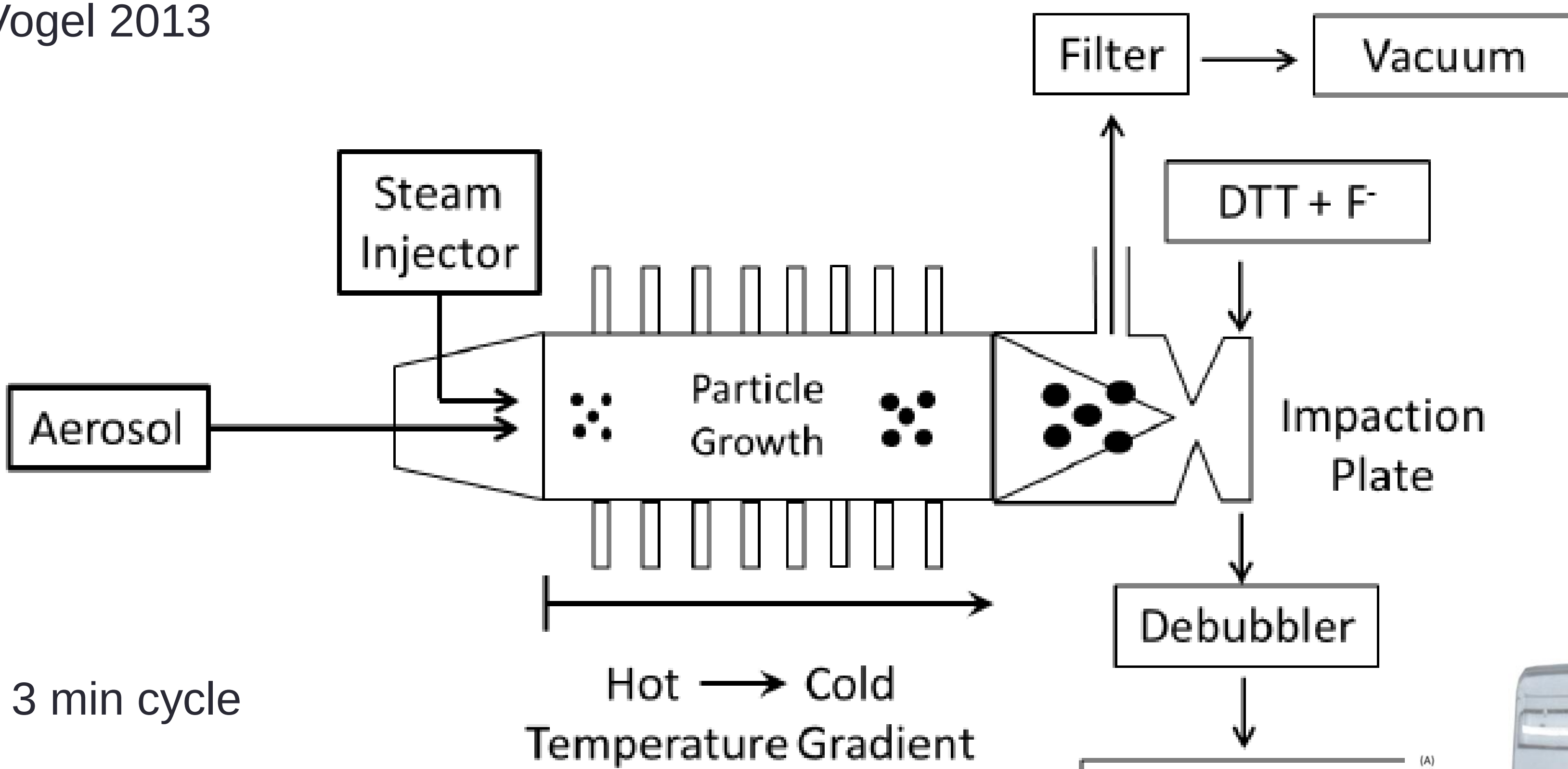
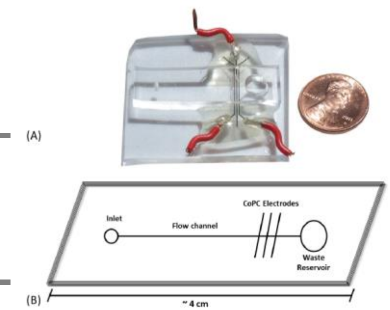


Fig. 4. Schematic of online $PM_{2.5}$ ROS measurement approach using a mist chamber particle collection system and fluorometric probe.



Vogel 2013

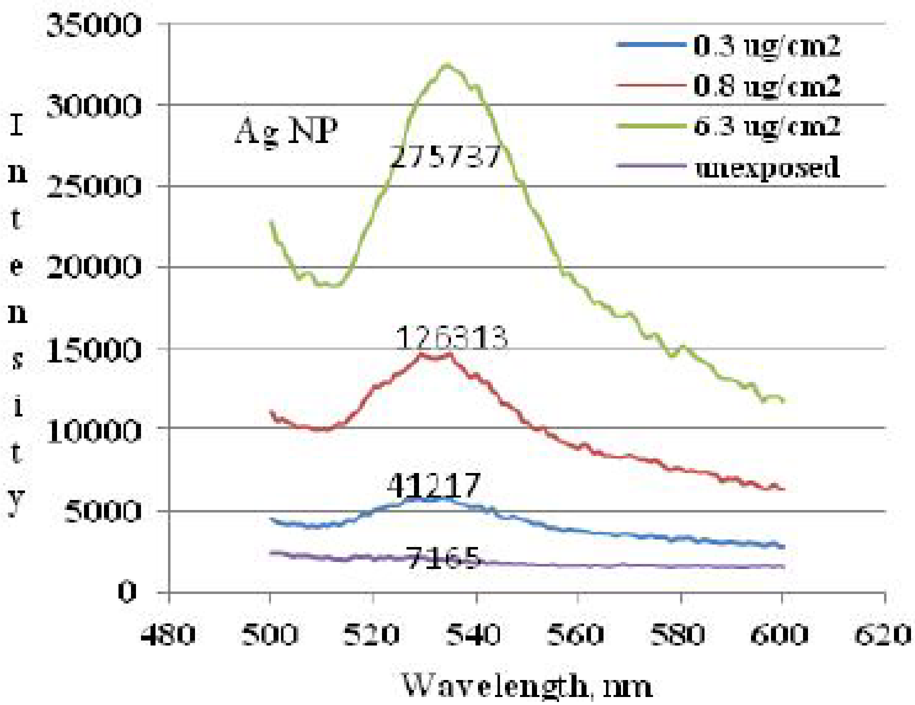


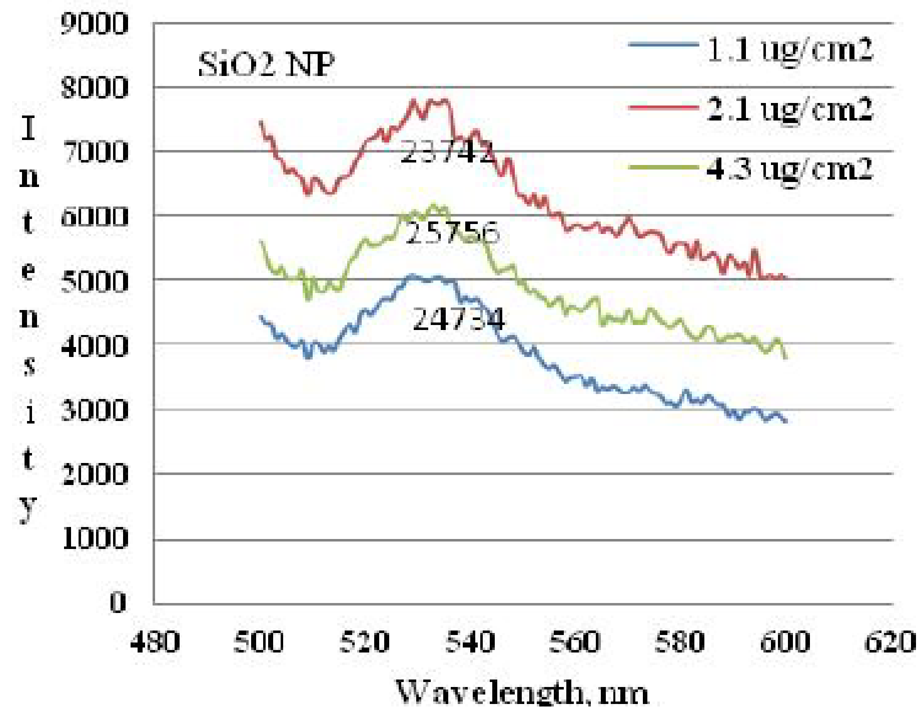


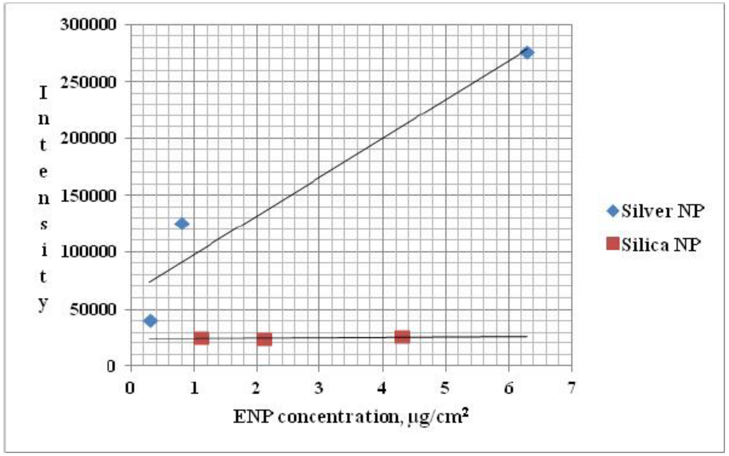
3 min cycle

Figure 6: Schematic of the PILS. A PM + steam mixture is cooled creating supersaturated conditions. Particles serve as condensation nuclei and grow into droplets large enough ($d_{ae} > 1$ µm) for collection at an impaction plate. DTT + F^- enters above the plate and a continuous liquid flow is pumped downstream for electrochemical measurement of DTT consumption.

Direct On-filter FLD detection







CONCLUSIONS

- Surface activity appears to integrate across multiple PCM parameters, including surface area
- Therefore, it is a critical parameter to capture, preferably in near-real time
- Additional parameter to SA
- The challenge- to develop the right technology

My Collaborators

Prof. P. Demokritou, HSPH

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Prof. J Isaacs, NEU

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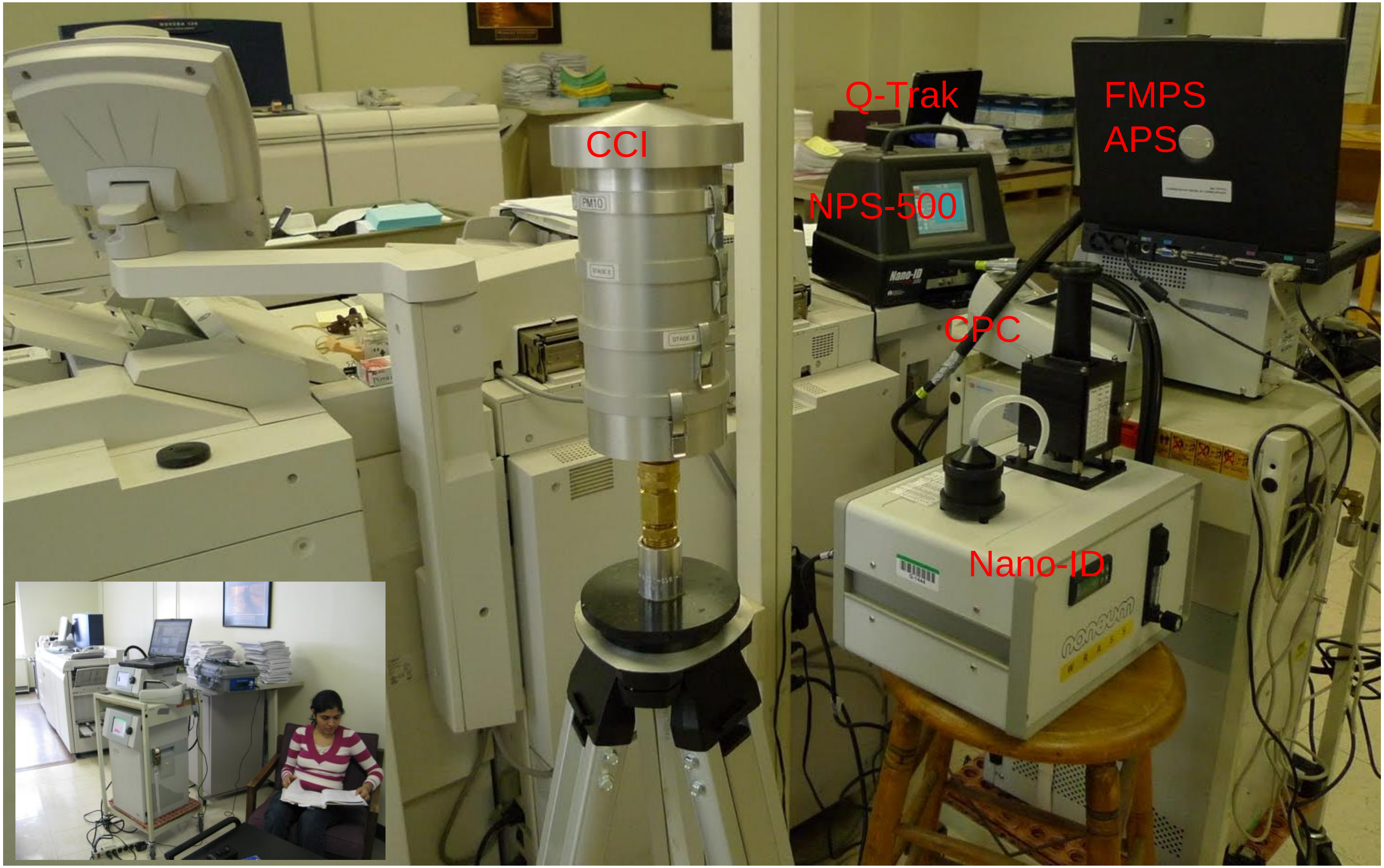
Prof. Redlich, Yale

Questions?

Monitoring NP Exposures, Instrumentation

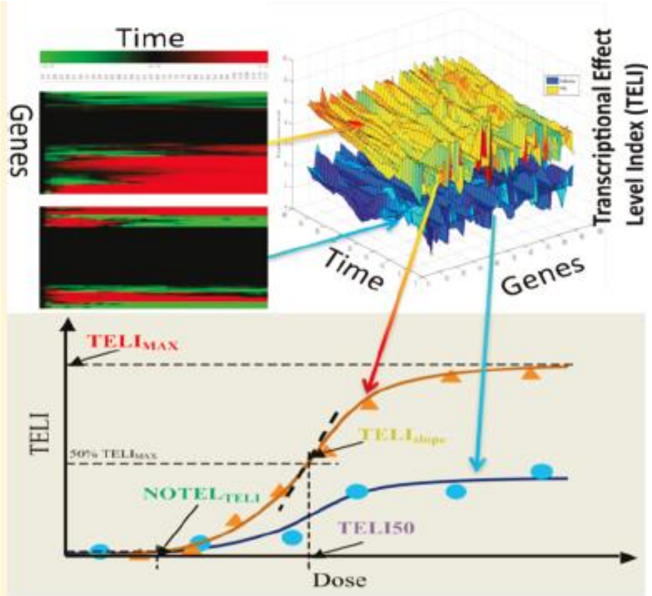


Personal Samplers

- Personal size selective impactors
  - Naneum Aerosol PS 300
  - Several miniaturized impactors

- Quazi Personal Real-Time Monitors
  - Philips NanoTracer (10-300 nm, TNC, SD)
  - DiSCmini (Matter-Aerosol Inc.)

BOD Correlates Well with TELI in *E-Coli*



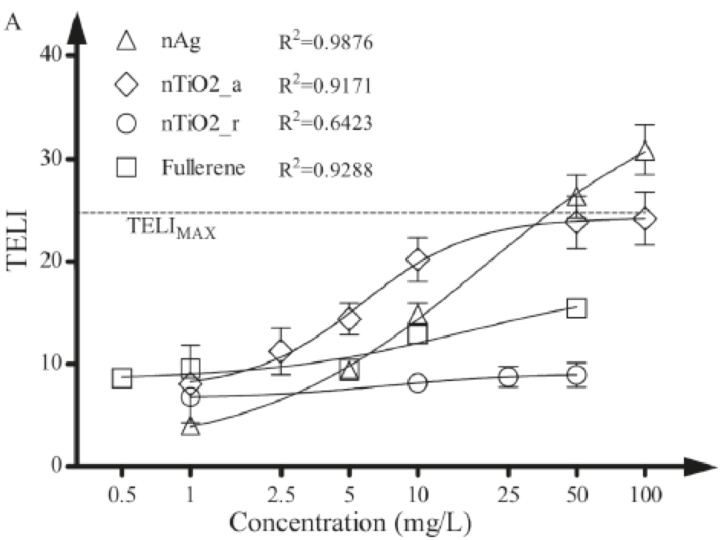


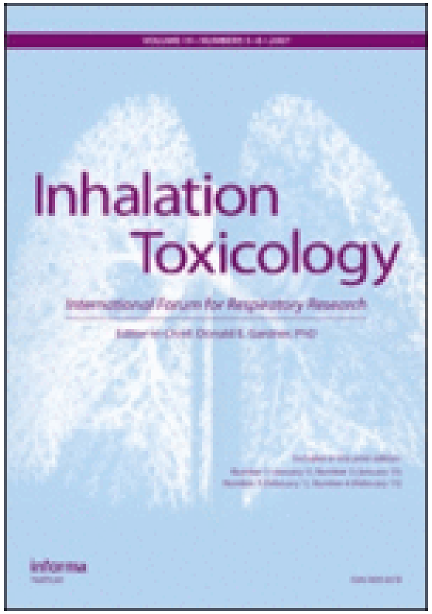
Table 2. Correlation Coefficients between TELI-Based Toxicity Endpoints with Other Toxicity EndPoints[a]

Correlation coefficient	$TELI_{MAX}$	$Slope_{TELI}$	TELI50	$NOTEL_{TELI}$
NOTEL (mg/L)	−0.51	−0.04	−0.20	0.40
	(−0.4)	(−0.4)	(−0.2)	(0.2)
EC50 (mg/L)	−0.82	0.39	−0.61	0.78
	(−1.0)	(0.4)	(−0.8)	(0.8)
BOD (μmol/L)	0.98	−0.63	0.80	−0.95
	(1.0)	(−0.4)	(0.8)	(−0.8)

[a] The values shown are pearson product-moment correlation coefficients, the values inside the parentheses are spearman's rank-order correlation coefficients.

Guo & Gu et al 2011 EST

dx.doi.org/10.1021/es200455p | *Environ. Sci. Technol.* 2011, 45, 5410–5417



Inhalation Toxicology

International Forum for Respiratory Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713657711

Evaluating the Toxicity of Airborne Particulate Matter and Nanoparticles by Measuring Oxidative Stress Potential - A Workshop Report and Consensus Statement

Jon G. Ayres [a]; Paul Borm [b]; Flemming R. Cassee [c]; Vincent Castranova [d]; Ken Donaldson [e]; Andy Ghio [f]; Roy M. Harrison [g]; Robert Hider [h]; Frank Kelly [i]; Ingeborg M. Kooter [j]; Francelyne Marano [k]; Robert L. Maynard [l]; Ian Mudway [m]; Andre Nel [n]; Constantinos Sioutas [o]; Steve Smith [p]; Armelle Baeza-Squiban [k]; Art Cho [n]; Sean Duggan [q]; John Froines [n]

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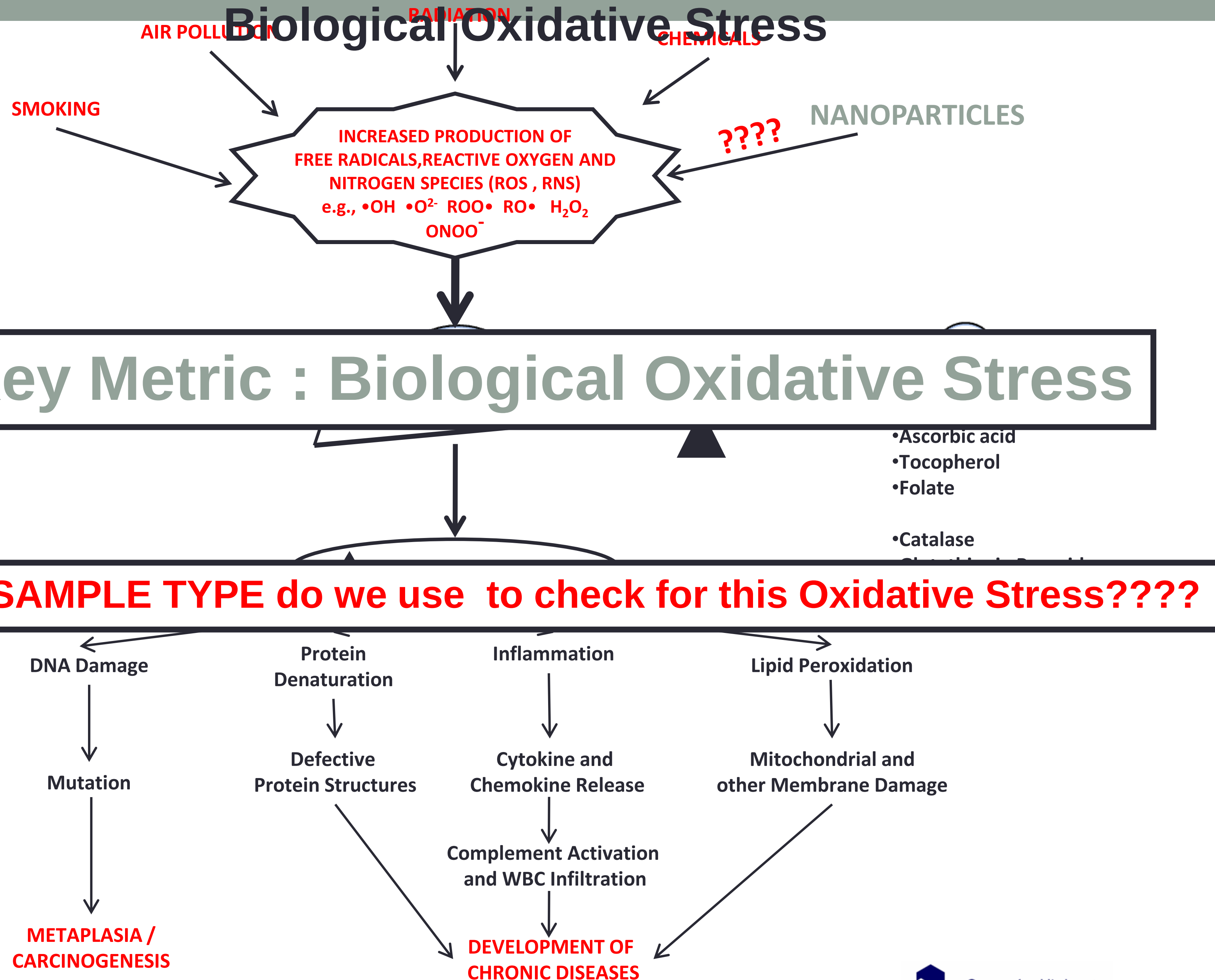
“Toxicity Screening tests for new nanomaterials products are urgently needed. Whilst recognizing that oxidative stress potential may not be predictive of all possible adverse outcomes, tests based upon oxidative potential maybe an invaluable tool for initial screening and classification of the relative biohazard of such materials.”

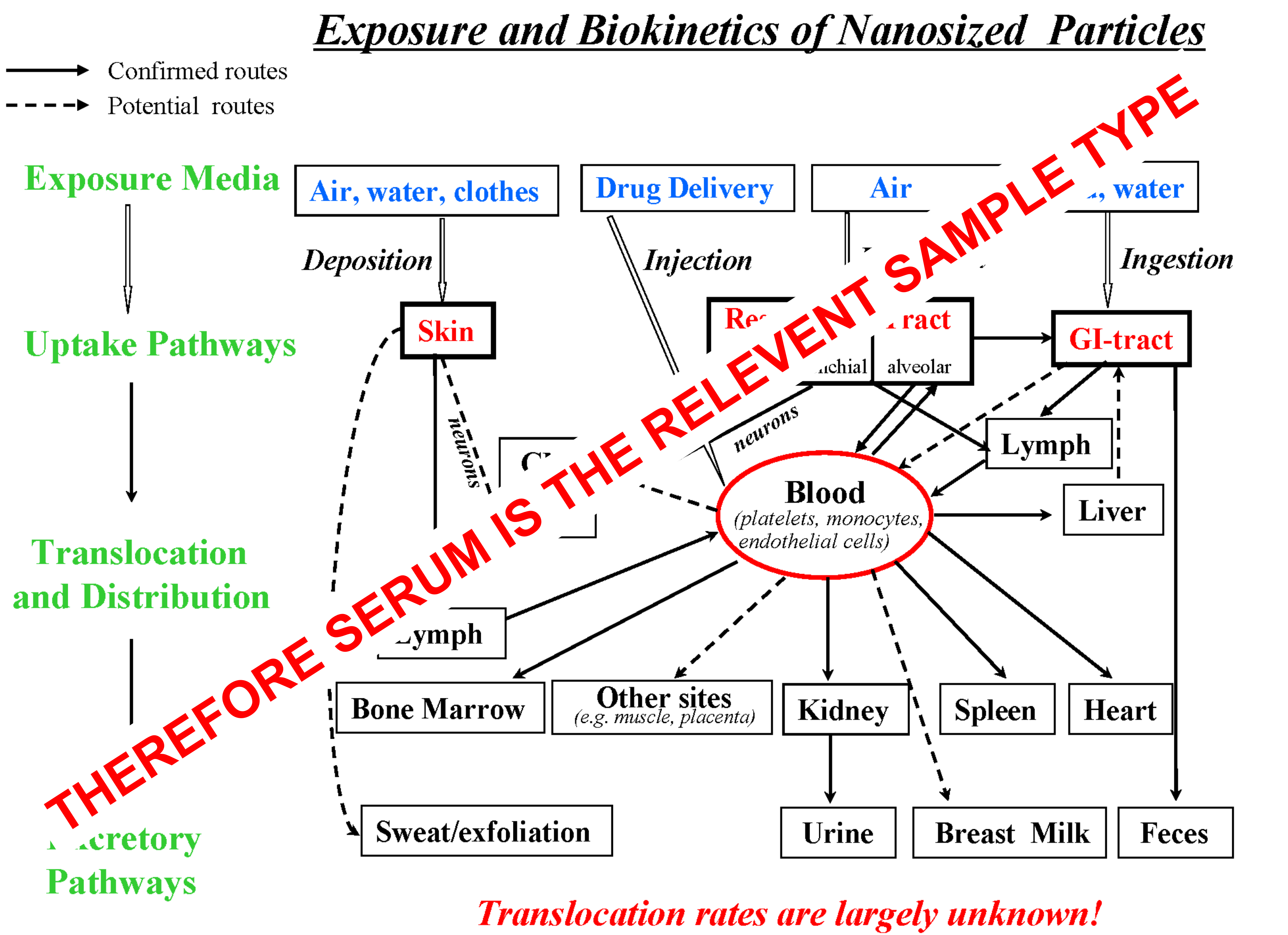
UMASS LOWELL

Center for High-rate Nanomanufacturing

Biological Oxidative Stress



Center for High-rate Nanomanufacturing



Exposure and Biokinetics of Nanosized Particles
Confirmed routes
Potential routes
Exposure Media
Uptake Pathways
Translocation and Distribution
Pathways
Air, water, clothes
Drug Delivery
Air
water
Deposition
Injection
Ingestion
Skin
alveolar
GI-tract
neurons
Lymph
Blood
(platelets, monocytes, endothelial cells)
Liver
Bone Marrow
Other sites
(e.g. muscle, placenta)
Kidney
Spleen
Heart
Sweat/exfoliation
Urine
Breast Milk
Feces
Translocation rates are largely unknown!
THEREFORE SERUM IS THE RELEVENT SAMPLE TYPE