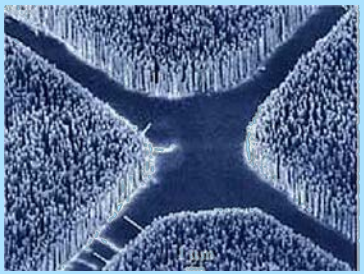
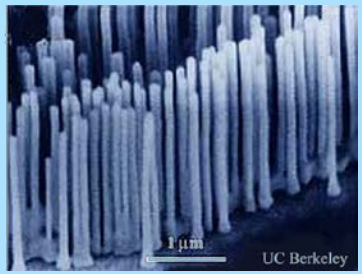


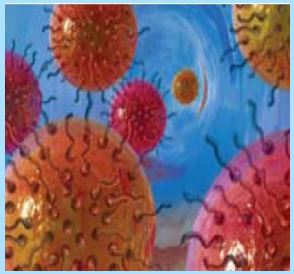
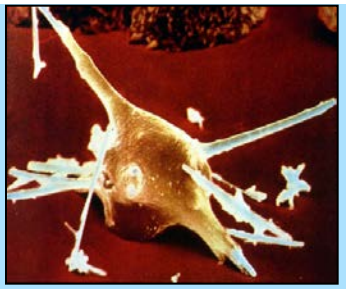

Linking Life Cycle Specific Exposures to Biological Impact of Nanomaterials

Philip Demokritou, PhD

Director, Center for Nanotechnology and Nanotoxicology

HARVARD SCHOOL OF PUBLIC HEALTH

CENTER FOR NANOTECHNOLOGY AND NANOTOXICOLOGY

http://hsph.harvard.edu/nano

Center for Nanotechnology and Nanotoxicology

at the Harvard School of Public Health

- **Focuses on Applications and Implications of engineered nanomaterials and nanotechnology**
  - **Mission:** Integrate material & exposure science and nanotoxicology risk assessment to facilitate science-based decision-making regarding nano-EHS.
  - **Current research activities:** Development of in-vitro and in-vivo toxicological screening platforms for ENMs, assess nano-EHS issues across life cycle of NEPs, safer by design development of ENMs and NEPs, Environmental Nanototechnology applications
  - **Industrial Partners:** BASF, Panasonic, Nanoterra, STERIS, Profector Life Sciences.
  - **International in nature:** Current collaborations with Federal Agencies, and Universities around the world (ETH, NTU- Singapore, MIT, SUNY, UMass, Northeastern Univ., NIOSH, CPSC, etc)

Website: http://hsph.harvard.edu/nano

Collaborators

Academic Collaborators

Harvard University Center for Nanoscale Systems

HARVARD School of Engineering and Applied Sciences

SUNY The State University of New York

Massachusetts Institute of Technology

UMASS

MARINA MANAGING RISKS OF NANOMATERIALS

ETH Eidgenössische Technische Hochschule Zürich

National Institute for Occupational Safety and Health NIOSH

Industrial Partners

Panasonic

BASF

nanoterra

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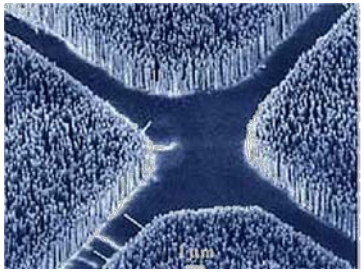
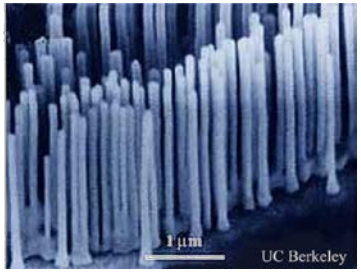
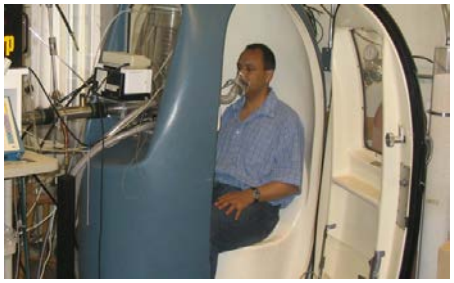
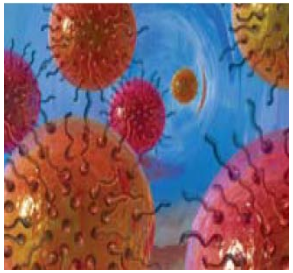
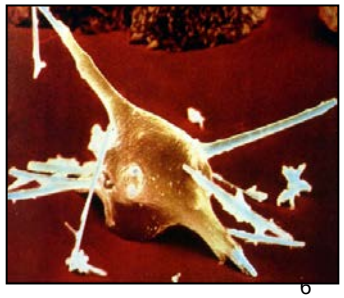
USDA/NIFA grant #: 2013-01614

NIEHS grant #: ES-000002

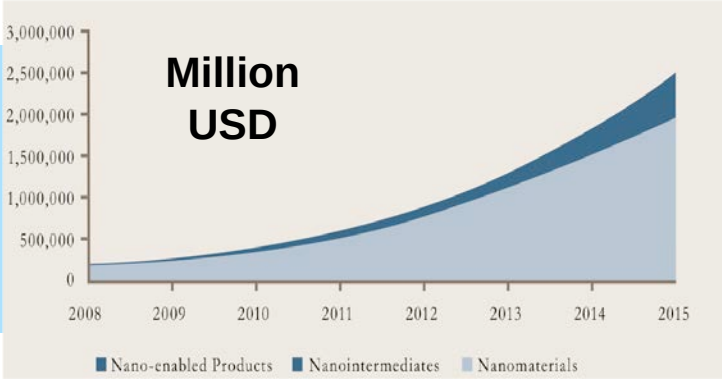
Presentation outline

- **Intro – 10+ year of Nano-safety research:** Progress, Knowledge gaps, “Scientific Sins”, failures and challenges ahead.
- **LC specific Exposures:** Human population exposures- Potential nanoparticle release across life cycle of NEPs
- **Linking LC specific exposures to biological impact:** Emerging integrated methods at the interface of exposure science and toxicology

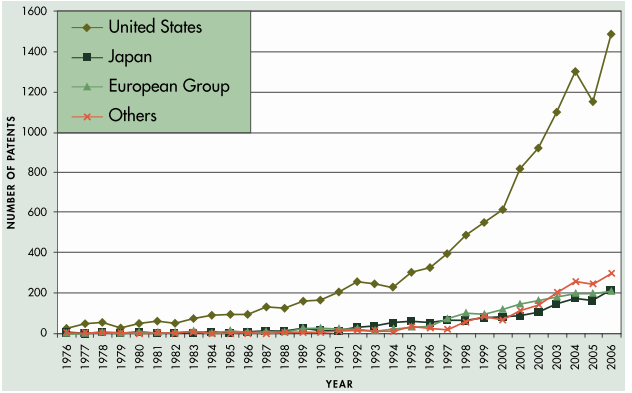



NT: Growing industry



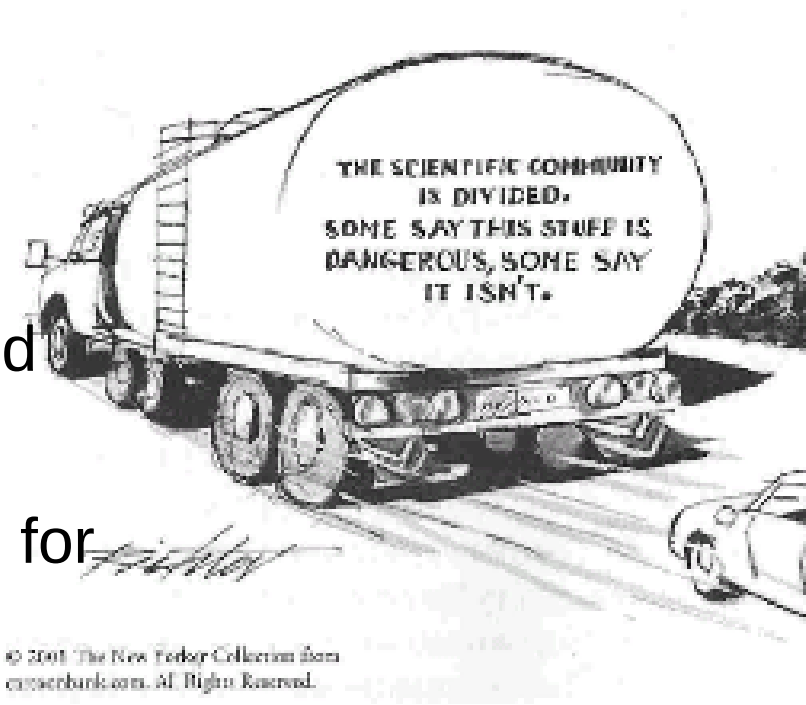
- NT is not longer at its infancy
- Key nanotechnology indicators: average 25% growth (2000 – 2008)
  - science citation index (SCI), patent applications, publications, R&D funding, etc
- **Commercialization** of NT: Slow
- There is still a **huge uncertainty** surrounding nano-safety
- **Nano-safety:** Key element for successful commercialization and sustainability of NT industry



10+ years of Nano-safety Research:

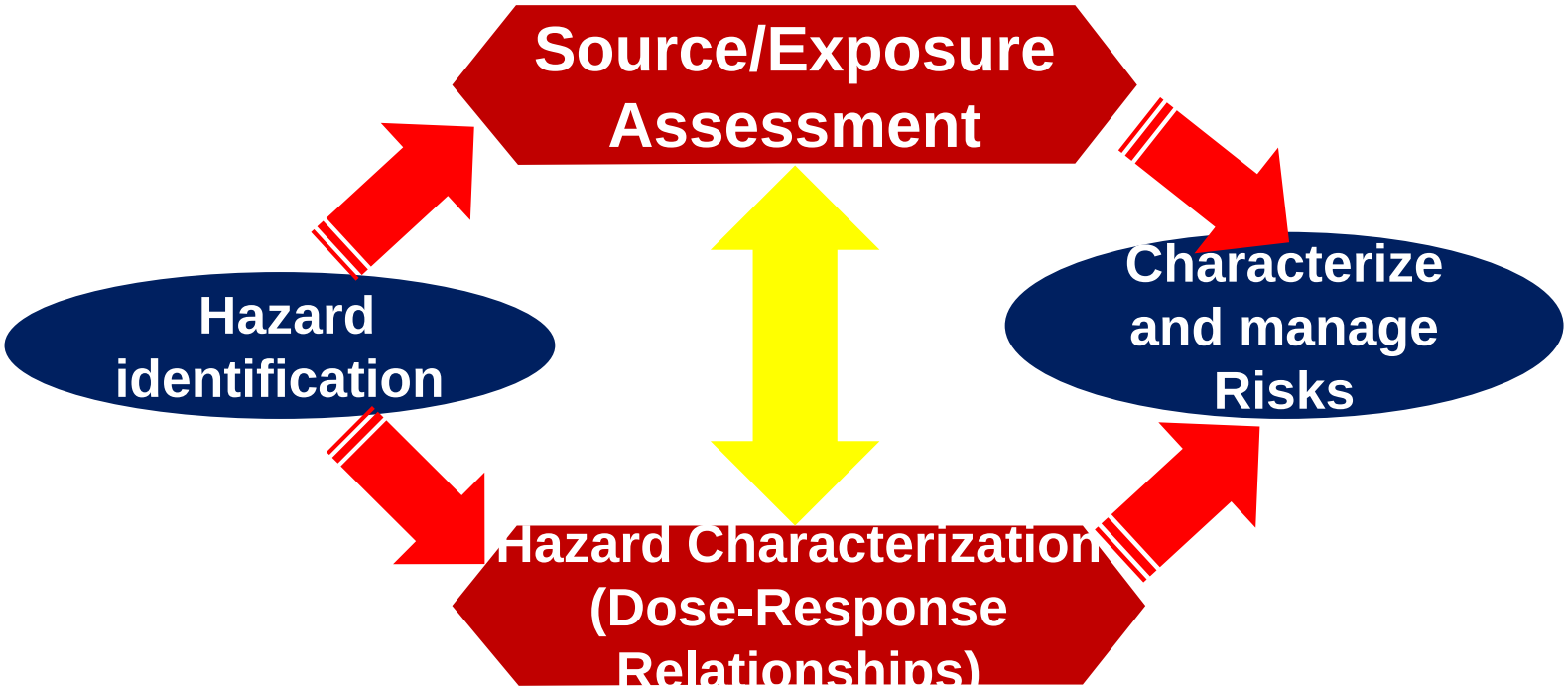
Knowledge gaps and critical issues

- **10,000 publications** in PubMed on toxicity of ENMs SINCE 2000, new nano-EHS journals
- **Billions $$** in nano-EHS research
- **Fact#1:** Nano-EHS is **lacking** behind
- **Fact#2:**Nano-EHS has become a negative force for commercialization for some sectors of NT (ie. CNTs)
- **Fact#3:** Paradigm shift for Risk perception for new technology in 21st century- Public considers any new technology as **unsafe** unless scientific evidence/data are provided



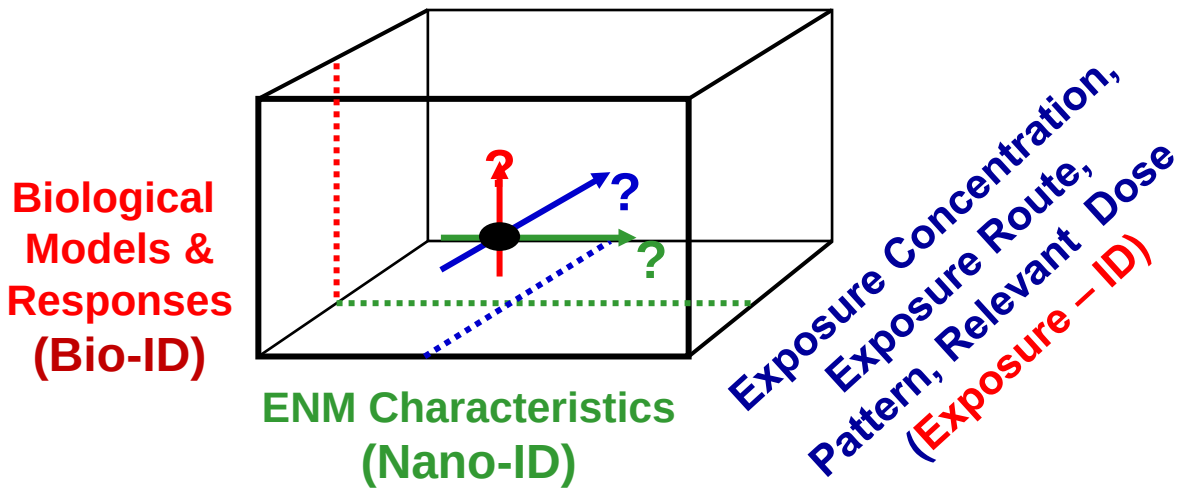
Nano Safety: Current Risk Assessment Paradigm (RAP) for ENMs

- **Same** as the one used for chemical risk assessment



- **Current RAP:** Is it **adequate** to address **nano-safety issues?**

The nano Risk space is 3 dimensional:
3 - IDs are needed to assess RISK



INFORMATION ON Nano-RISK HAS EXPANDED SINCE 2005…

… BUT HAVE THE ISSUES EVOLVED SUBSTANTIALLY?

Nano-ID Challenges: Too many intrinsic properties to consider (1/3)

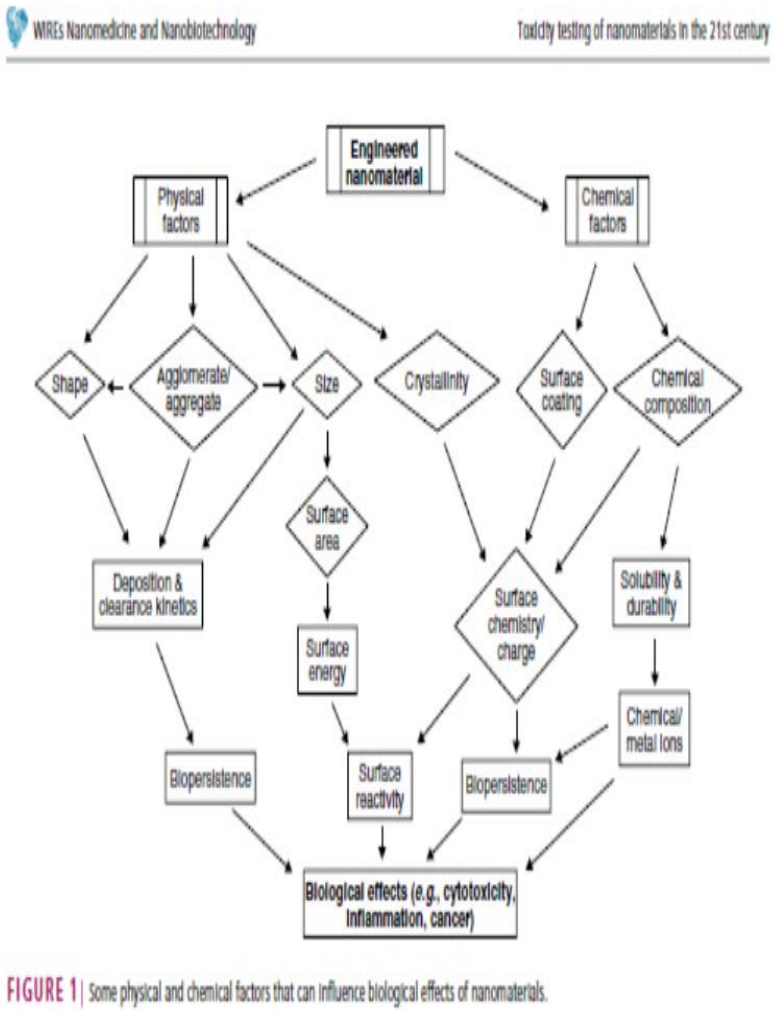
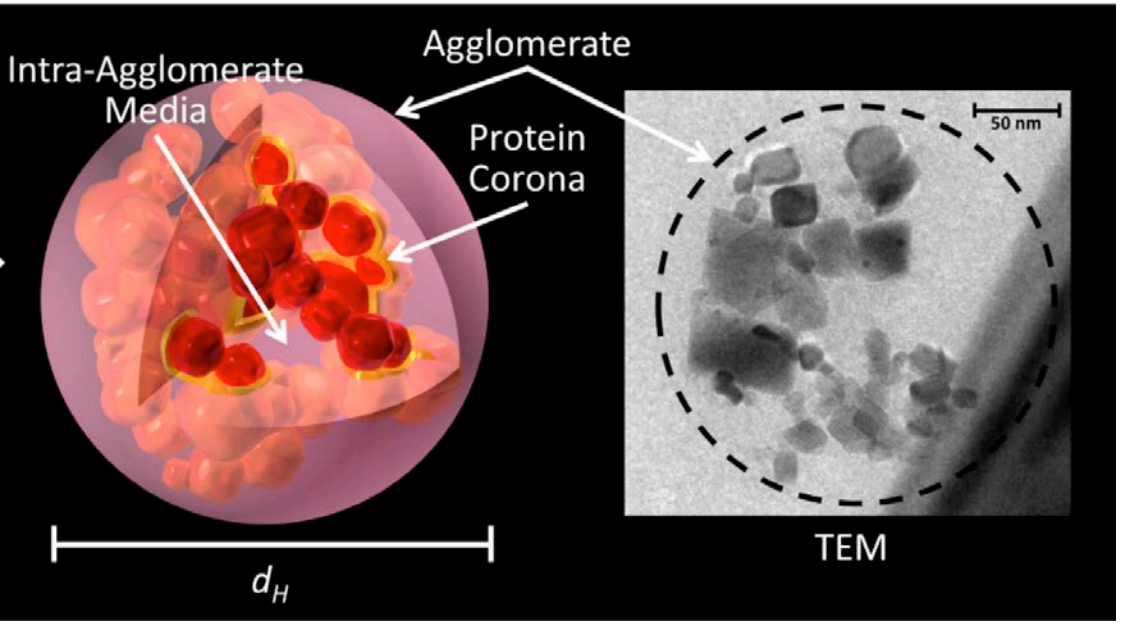


FIGURE 1 | Some physical and chemical factors that can influence biological effects of nanomaterials.

(David Lai, 2012)

- ENMs are far more complicated in regard to property characterization
- **Nano-ID:** Many intrinsic properties (size, shape, agglomeration stage, crystallinity, charge, surface chemistry, etc
- Gazillion of property combinations

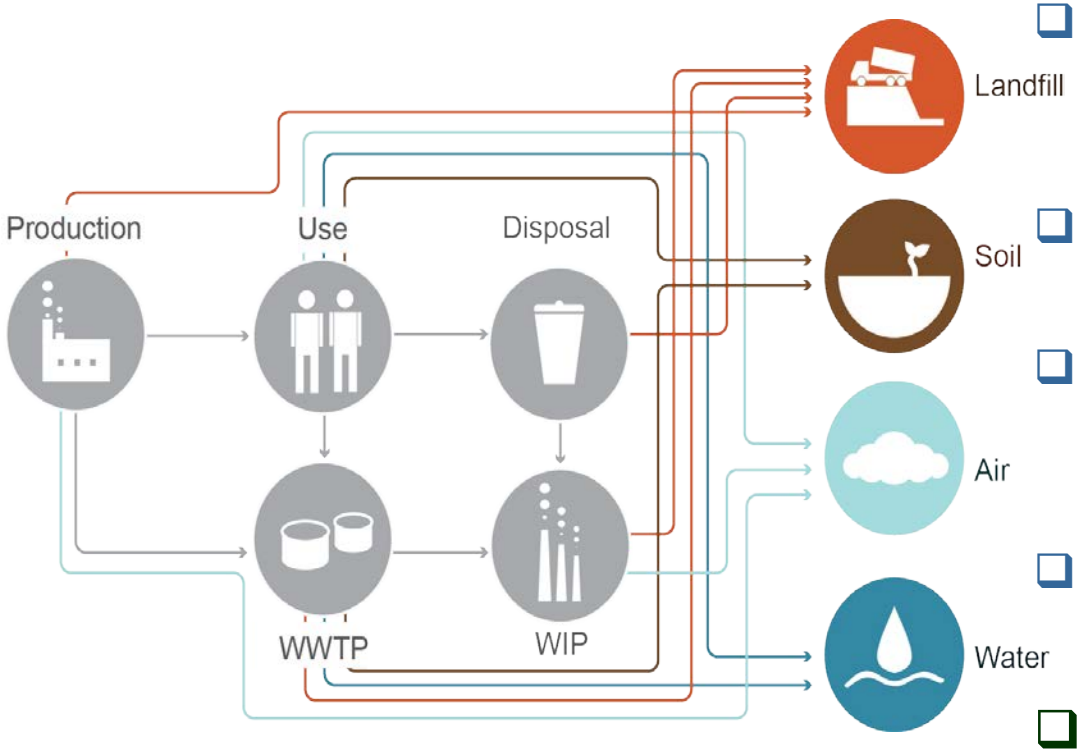
Nano-ID Challenges: Extrinsic properties (2/3)



- **MEDIA properties, not solely the INTRINSIC p-c-m properties of ENMs, affect agglomeration[1], bioactivity and fate and transport in biological media**
- Protein corona has implications on agglomeration potential and particle to cell interactions[2,3]

[1]*Pyrgiotakis et al., Langmuir, 2013*
[2]*DeLoid et al. Nature Comm., 2014*
[3]Lynch et al., Nature Nanotech, 2009

More Nano-ID Challenges:
ENM property changes across value chain and life cycle



- ENM properties change in both value-chain, and **across life cycle of NEPs**
- Limited data on ENM release dynamics across LC
- Fragmentary exposure data for both env. media and human population
- Current RAP focuses on properties of raw ENMs
- Regulators are asked to decide on nano-EHS matters based on the tox properties of raw ENMs

Keller and Lazareva, ES&TL, 2014

Nano-safety research: “SINS”

“No, no, that’s not a sin, either. My goodness, you must have worried yourself to death.”

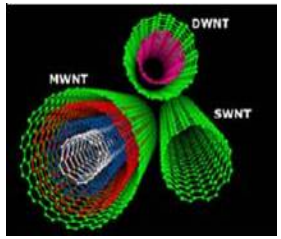
Current Risk Assessment Paradigm:
Did we ask the right questions?

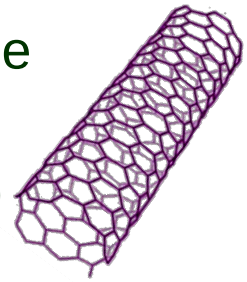
at plausible **DOSES** and **EXPOSURE** conditions,

- **"HAZARD IDENTIFICATION" : Can the material cause an adverse health effect?**
- **"HAZARD CHARACTERIZATION: What effects? Under what EXPOSURE concentration, DOSES, and time?**
- **"RISK: We need "real world" EXPOSURE in addition to hazard characterization data to determine RISK?**

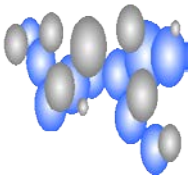
SIN#1: Too much attention and $$ was spent on hazard identification

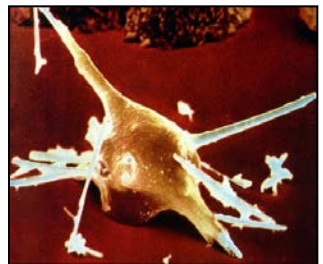
- **ENMs: Unique Physico-chemical properties**
  - Extraordinary small – similar size as UFP
  - More particles per unit mass.
  - Greater surface area per unit mass.
  - High surface reactivity
  - Some ENMs have asbestos like physical properties (large aspect ratio, insoluble, bio persistent, etc).
  - New size dependent material properties (Quantum effects)





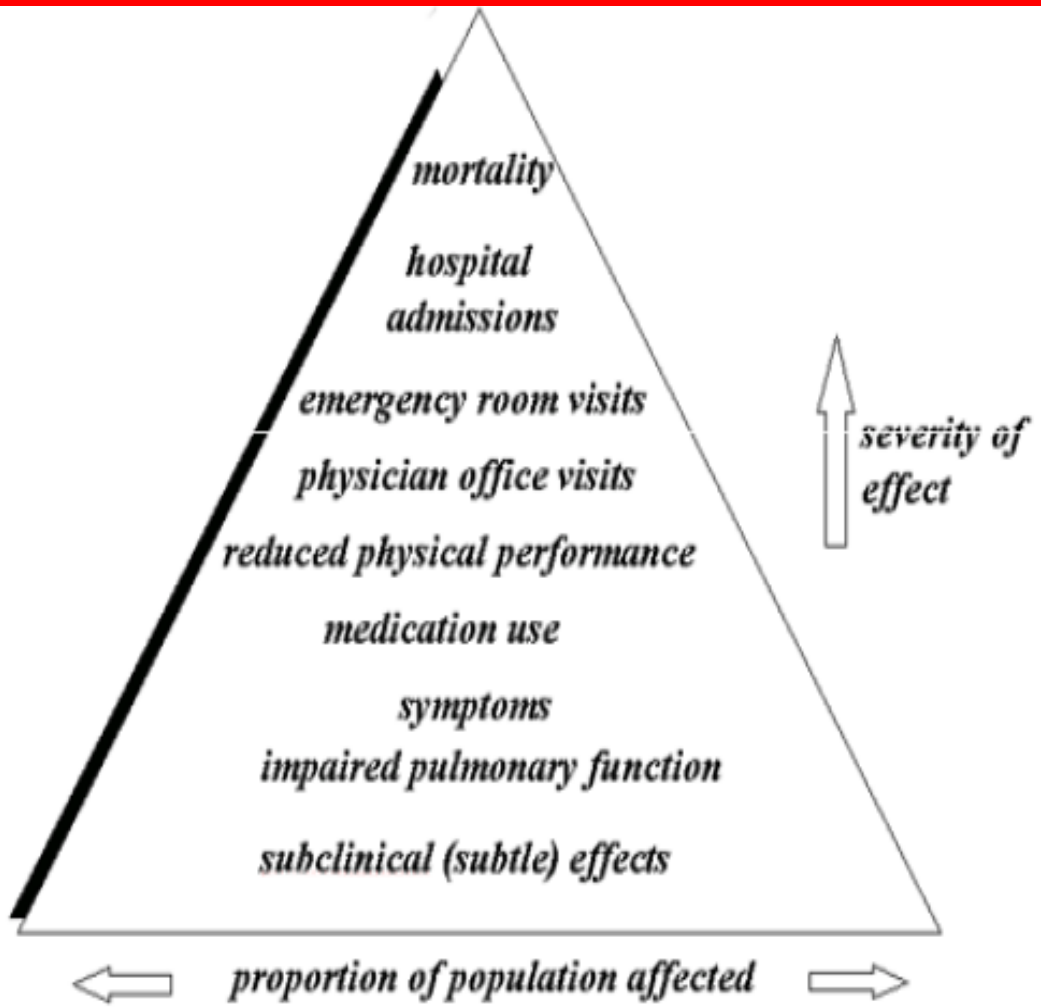
- **High mobility in both biological and environmental media**
- **Penetrate biological barriers (Exhibit novel translocation pathways: i.e via Olfactory neurones. Elder, 2006)**





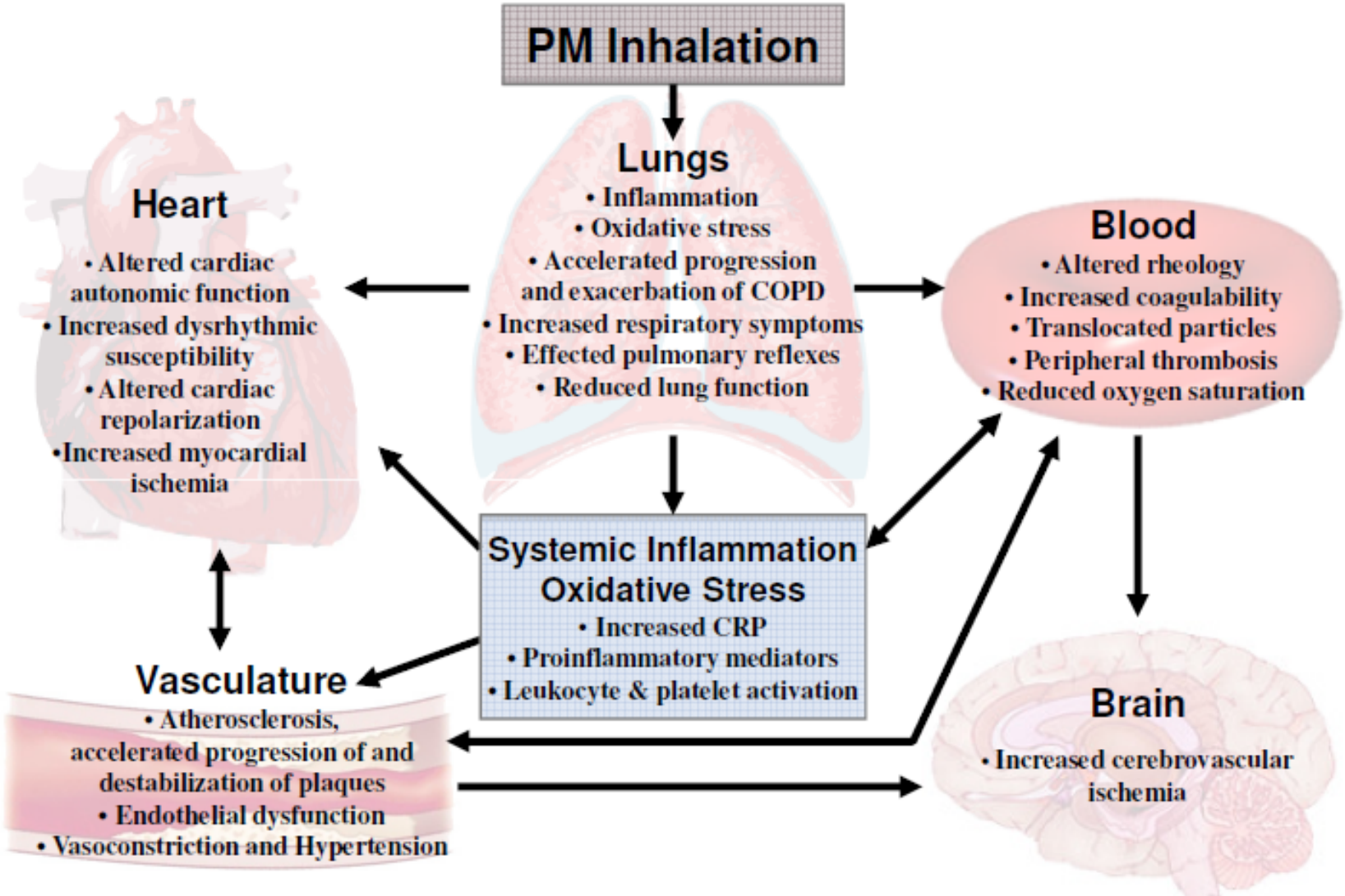
Do we have reasons to believe ENMs can be hazardous?

Historic epi and tox data on Ambient PM Health Effects



Lessons learned for complex mixtures related to ambient PM?

…Multiple mechanistic pathways, complex interactions and interdependencies…..



More "SINS" ….
Plausible doses, physiologically relevant exposures and exploratory biology

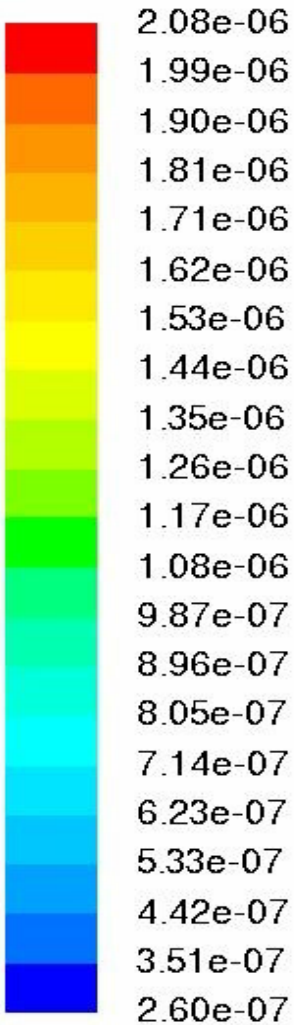
- **Plausible and physiologically relevant doses:** Unfortunately, nanotox literature is flooded with implausible doses which are not based **on "real** world" exposures.

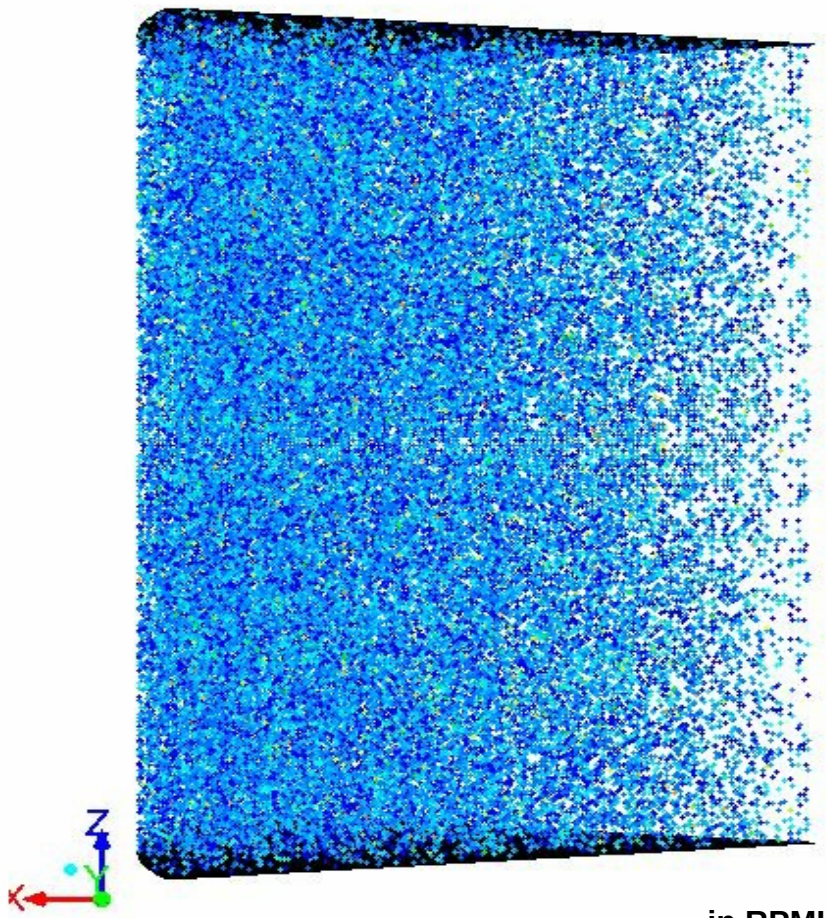
- There is no "sin" in beginning with a high dose- this is part of hazard characterization…. but there is much mischief in continuing to do so

- We need to differentiate between **biological outcomes/ exploratory biology** Vs **adverse health effects**

- **How about dosimetry?** There is a lack of standardized, easy to use, and validated tools and methodologies to bring in-vitro and in-vivo doses to the same scale despite the growing evidence of its importance in hazard ranking.

In-vitro dosimetry- Effect on hazard ranking





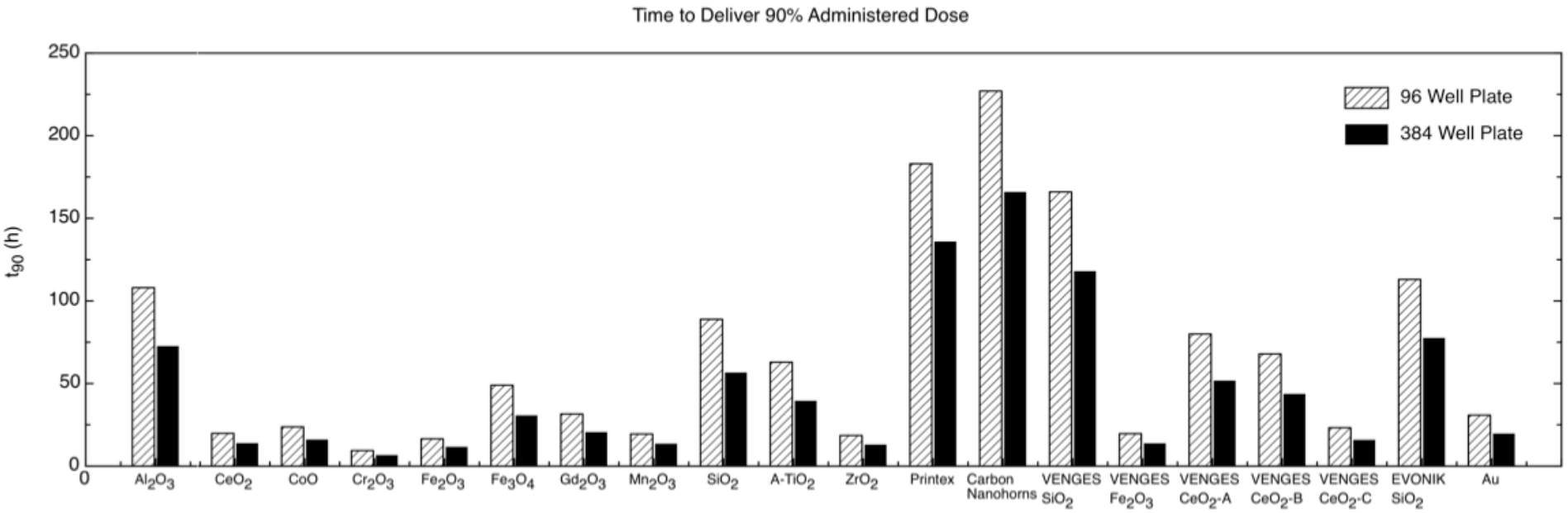
Time: 0.0 hour

[1]Deloid et al. in revision

CeO_2 (d_{BET}= 5.4 nm) in RPMI/BSA (d_H = 982 nm, ρ_{EV} = 1.472 g cm^{-3})

Particle Traces Colored by Particle Diameter (m) (Time=1.0000e-06) Sep 05, 2014
ANSYS FLUENT 14.0 (3d, dp, pbns, lam, transient)

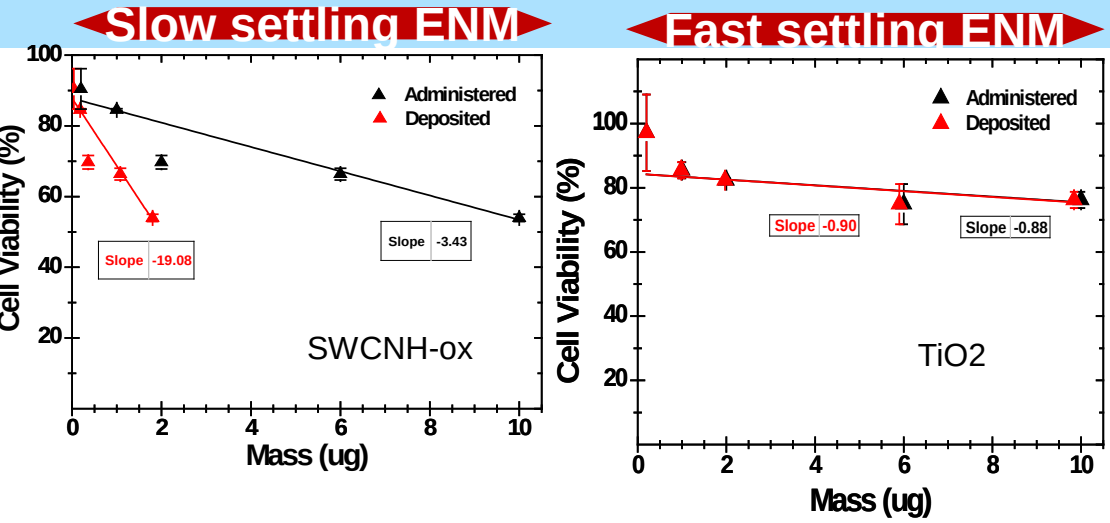
Effective Density and Agglomeration potential Influence Particle Delivery to Cells and dose (3/4)



Differential mobility & settling rates- dosimetry has to be considered in in-vitro nanotoxicology studies for accurate hazard ranking

(Cohen et al. , PFT, 2014)

RESULTS: Implications of dosimetry on in-vitro hazard ranking for low aspect ratio ENMs(4/5)



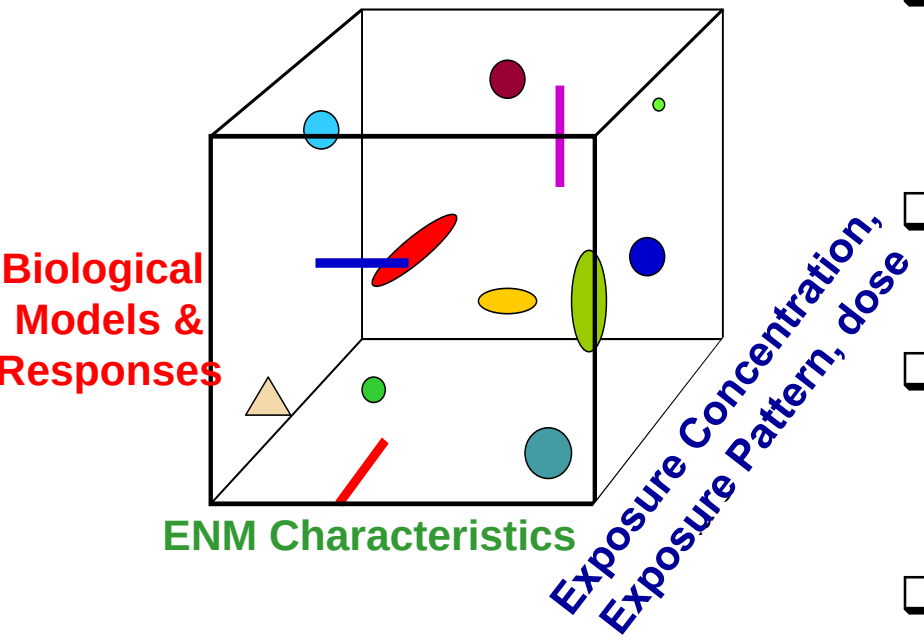
Toxicity Endpoint	Cell Viability (% Live Cells per unit mass)			
Nanomaterial Label	Slope, Admin.	Slope, Delivered	Slope Ratio, Delivered/ Admin.	IC75 (Delivered Dose)
SWCNH-ox	-3.30	-18.31	5.55	0.58
N110	-1.92	-10.01	5.21	1.22
Printex-90	-2.74	-8.05	2.94	2.73
TiO_2 P25	-0.88	-0.90	1.02	17.24
CeO_2	-1.15	-1.51	1.31	11.24
Ni Inco	-4.37	-7.41	1.69	1.61
MnOx PALAS	-2.57	-4.49	1.75	4.35
nAg	-1.86	-2.46	1.32	4.21

(Pal et al. Nanotoxicology, 2015

- Marked differences between the slopes of administered and delivered dose-response curves were observed for "slow-settling" ENMs,
- Negligible corresponding differences for "rapidly-settling" ENMs.
- Hazard ranking change when delivered dose is considered.

Nano- RISK 3D model

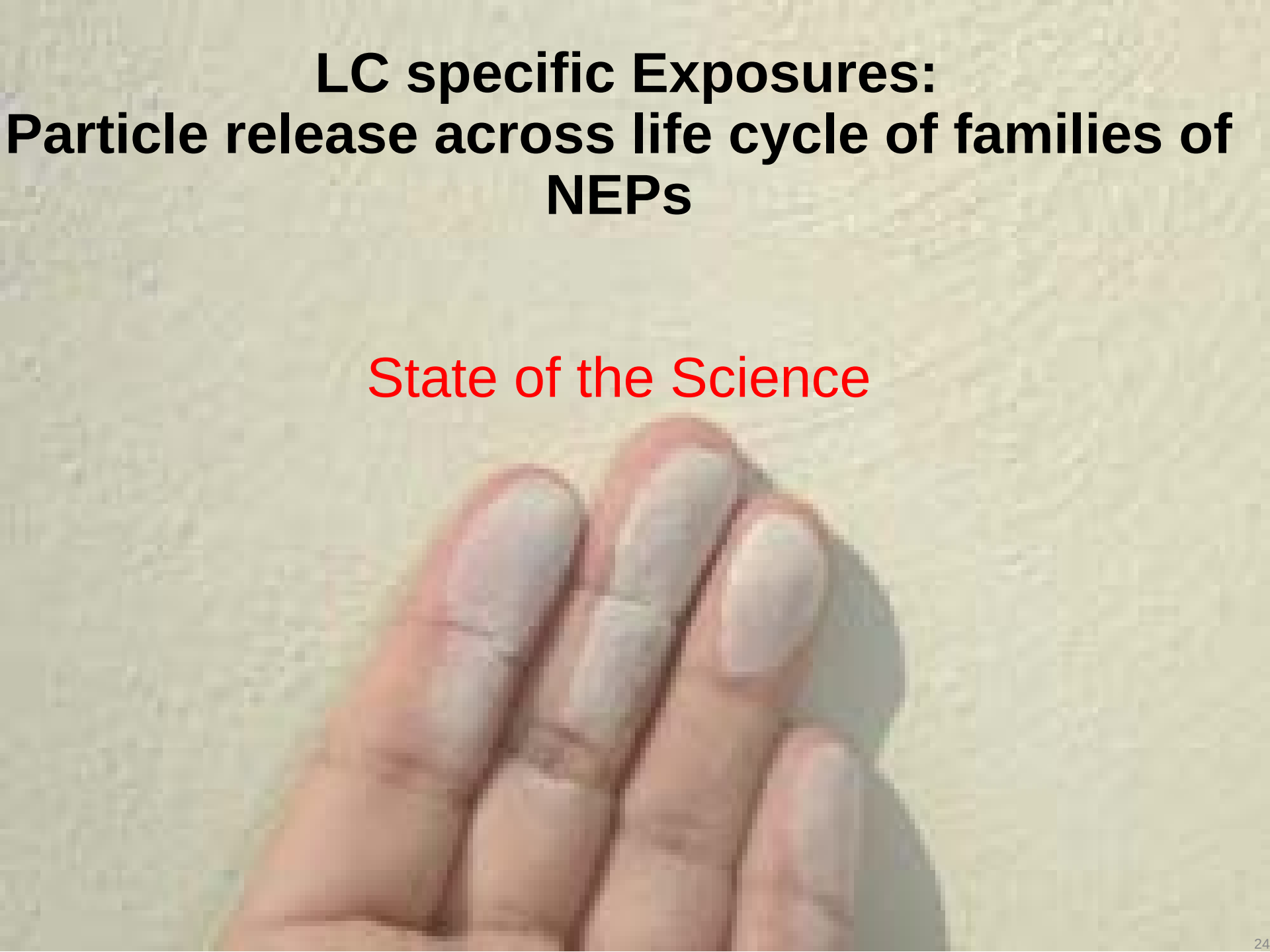
DO WE HAVE A SYSTEMATIC UNDERSTANDING?
OR WE JUST GENERATED POINTS OF INFORMATION



- Progress has been made in understanding key ENM toxicity pathways at molecular and cellular level
- Major knowledge gaps exist preventing us from a systematic understanding of rules of nanotoxicology
- **Fact #1:** There is still a **huge uncertainty** surrounding nano-safety
- **Fact#2:** Current RAP and methodologies are not adequate to assess RISKS across life cycle of NEPs
- **Fact#3:** Major knowledge gaps prohibit science based regulations
- Population Exposure data across life cycle of NEPs are fragmentary

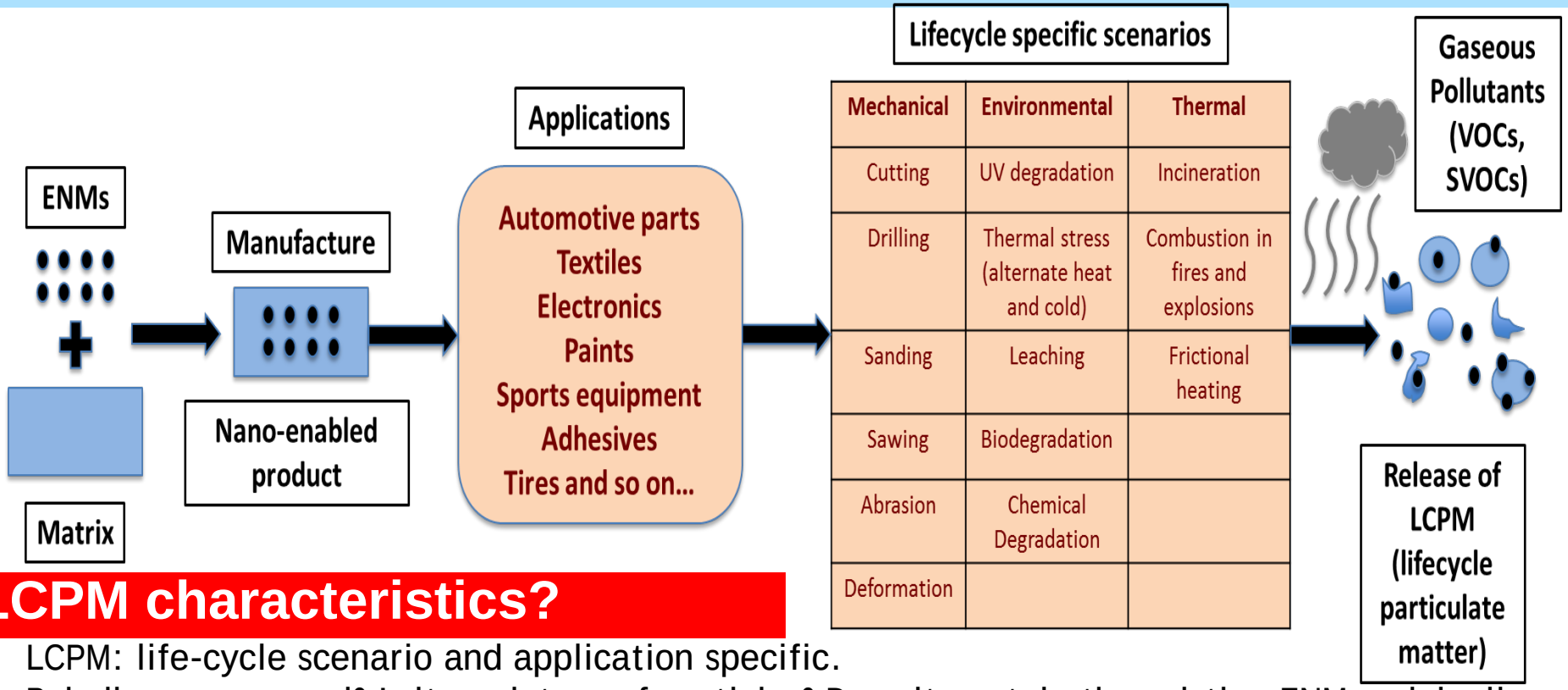
LC specific Exposures: Particle release across life cycle of families of NEPs

State of the Science



Particulate Matter released across life – cycle of NEPs (LCPM)

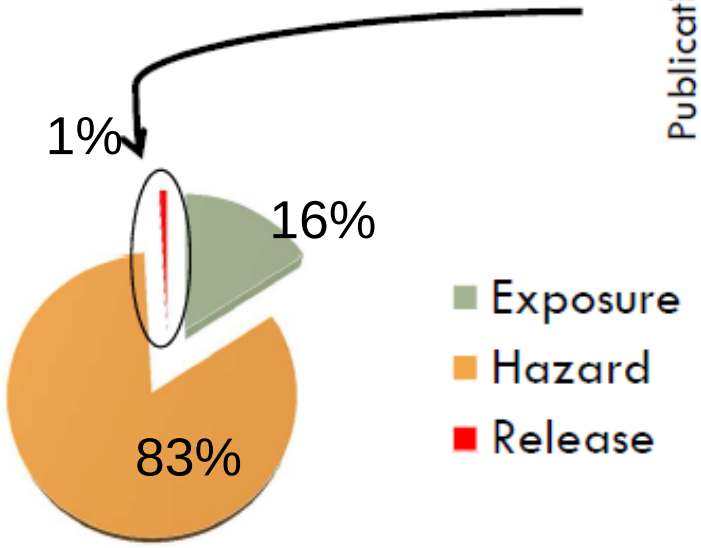
Pal et al., Tox. Sci.,,2015

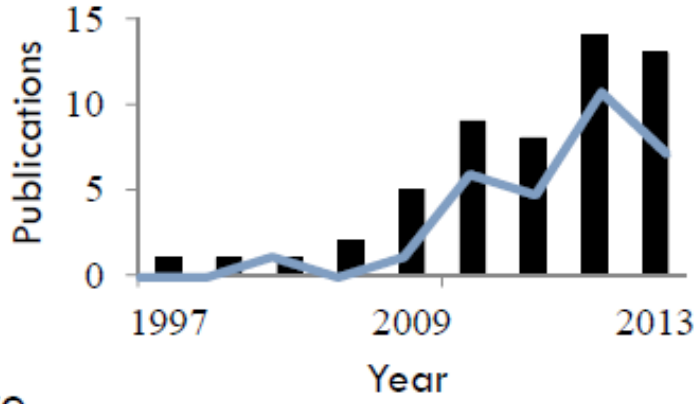


LCPM characteristics?

- LCPM: life-cycle scenario and application specific.
- Polydisperse aerosol? Is it a mixture of particles? Does it contain the pristine ENMs originally used in NEP synthesis?
- LCPM may be accompanied by release of gaseous co-pollutants depending on the specific lifecycle scenario (e.g., frictional heating, incineration, etc.)?
- Physicochemical and toxicological properties of LCPM can be significantly different from the pristine ENMs used in the synthesis of NEP?

Limited but emerging research on LCPM release for families of NEPs



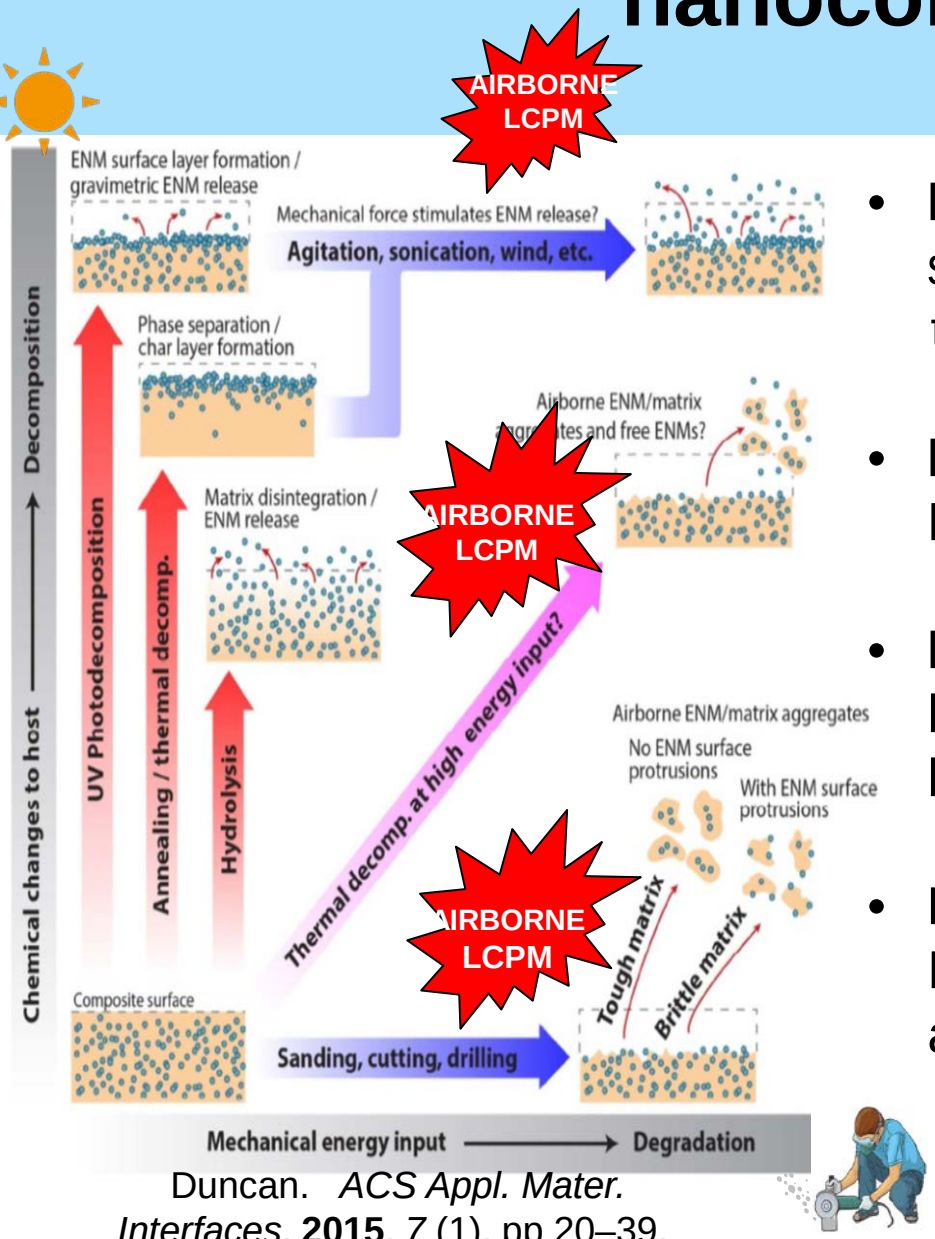


Froggett et al, Part Fibre Toxicol 2014, 11:17

*~**54** studies focused on inducing, detecting & characterizing release from solid nanocomposites*

Major drawback: Lack of standardized, reproduciple LC specific nanorelease methodologies

LC specific Release Mechanisms for polymer nanocomposites



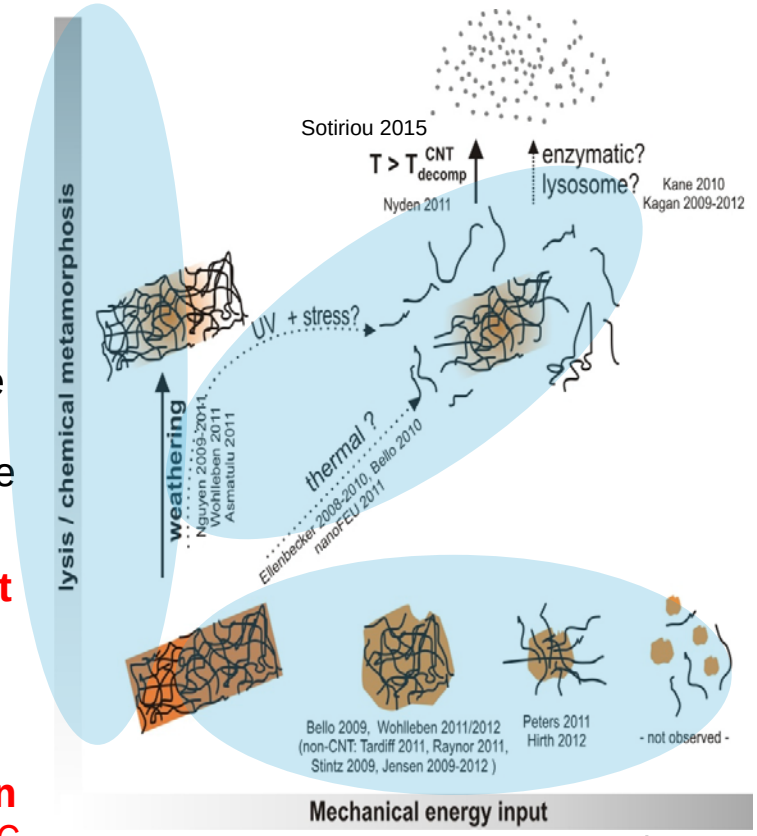
- **LCPM Release:** 3 main "lysis" LC scenarios- Mechanical, chemical and thermal
- **Mechanism/dynamics of LCPM release:** Life cycle scenario/application specific
- **LCPM properties**: primarily determined by LC scenario, then by host matrix, least by ENM filler
- **LCPM** may or may not contain the raw ENMs, its usually polydisperse in nature and gaseous co-pollutants may co exist

Duncan. *ACS Appl. Mater. Interfaces*. **2015**, *7* (1), pp 20–39.

Example: CNTs embedded in thermoplastics

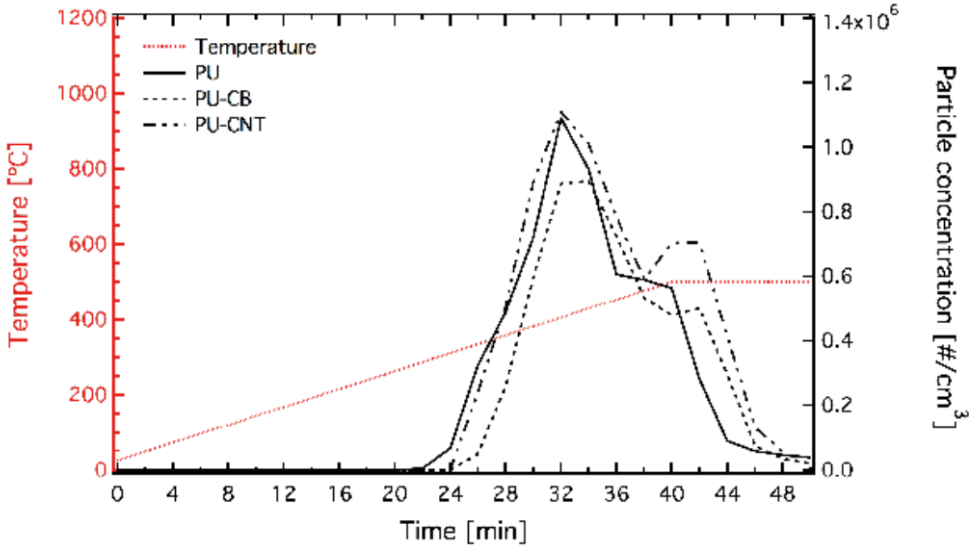
- Thermoplastics (TPs) are used in sport goods, automotive and aerospace applications
- Photochemical, mechanical and thermal degradation scenarios of TPs with CNTs [1,2]
- Networks of CNTs remain intact after **photochemical degradation** of the matrix
- **Mechanical stress** alone does not release free CNTs, but large micron scale particles with protrusions for brittle matrix.
- **Thermal degradation** at 2 different temperatures (500ºC and 800ºC) did not release CNTs (free or bound) into the released aerosol, however, CNTs were surface-bound in the brittle matrix of the residual ash at 500ºC.
- **P-c-m properties of "raw" CNTs are different than those of "released" LCPM. Tox properties of released PM are most likely different as well**
- **Current Risk Assessment Paradigm is based on "raw" ENMs.** Need to take into consideration LC Specific exposure scenarios

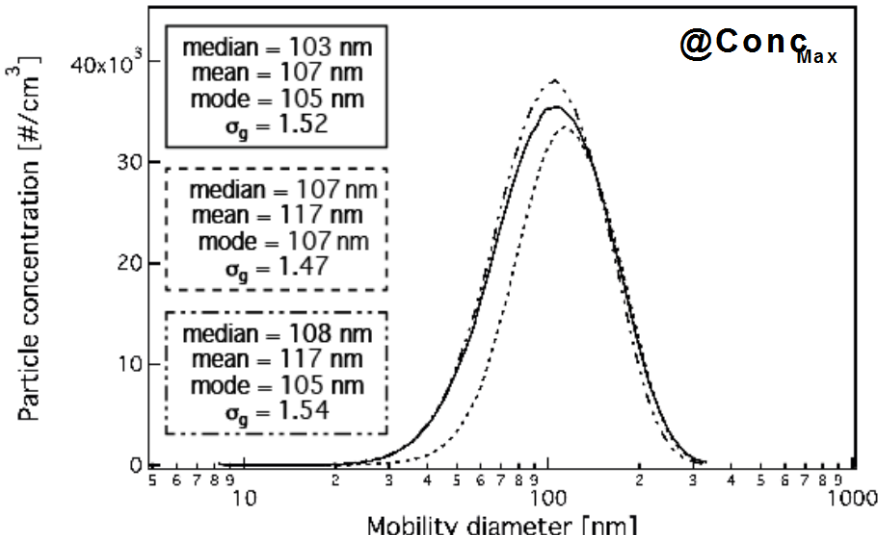
(2011-2013: *Nanorelease* project with ILSI + US-EPA)



[1] Wohlleben et al., *Nanoscale* **5**, 369 (2013).
[2] Sotiriou et al, Es Nano, 2015

Example#2: Thermal decomposition/incineration of polymer nanocomposites:
Does the presence of nanofiller influence the released aerosol concentration & size? (1/4)





- PU-based NEPs
  - Pure and with two different nanofillers(CNTS and CB)
- Route 1 at 500 ˚C

RESULTS

- No effect on released aerosol concentration and size due to the nanofiller presence
- Host polymer dictates the released LCPM

Sotiriou et al., ES: Nano, 2015

Does the presence of nanofiller influence the chemistry of the released aerosol for carbon based NEPs? (2/4)

- **Host polymer matrix dictates the chemical composition of the released aerosol**
- **TD of polymers generates various gaseous organic byproducts[1,2]**
  - Polydispersed aerosols, Aromatic, aralkyl, cycloaliphatic gaseous co pollutants

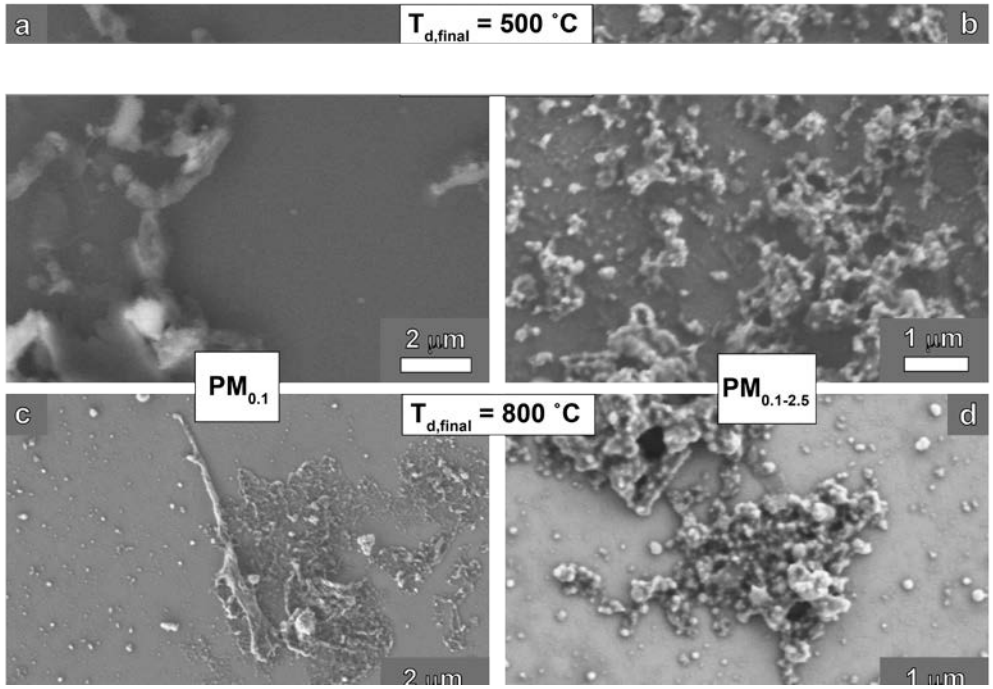
	500 ˚C		800 ˚C	
	EC (%)	OC (%)	EC (%)	OC (%)
PU	0.8	99.2	0.8	99.2
PU-CB	0.8	99.2	0.8	99.2
PU-CNT	0.8	99.2	0.8	99.2

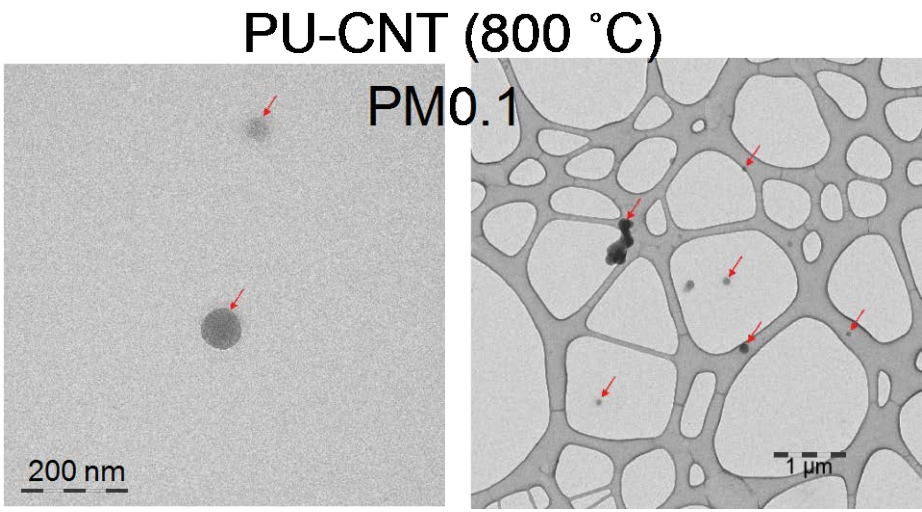
[1] Matuszak, Frisch. *J. Polym. Sci. Pol. Chem.* **11**, 637 (1973).

[2] Sotiriou et al., ES: Nano, 2015

Is there "nanofiller" (CNT) release in the air (3/4)?

- **PU-CNT**





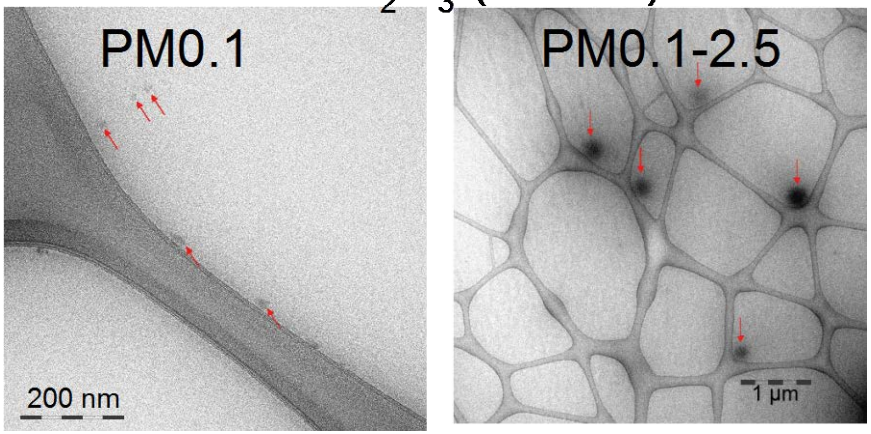
- No CNTs in the released aerosol for both size fractions and TD temperatures

Sotiriou et al., ES: Nano, 2015

Is there a "nanofiller" release in the air (4/4)?

- Is there Fe in the released aerosol?
  - 0.004 % Fe for $T_{d,final}$ = 500 ˚C
  - 0.026 % Fe for $T_{d,final}$ = 800 ˚C

PE-Fe_2O_3 (800 ˚C)

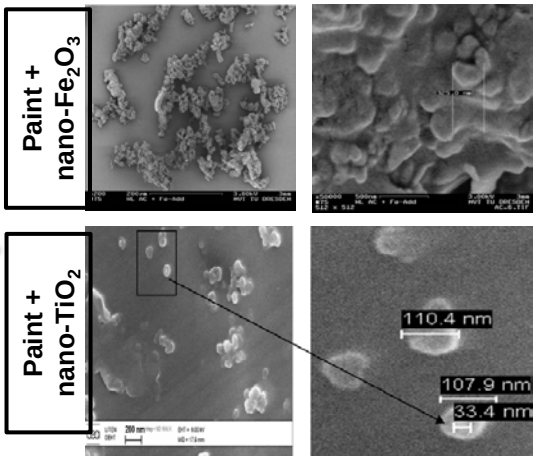


- Release of nanofiller in the air is more likely for the case of inorganic nanofillers (Me/MeOx)

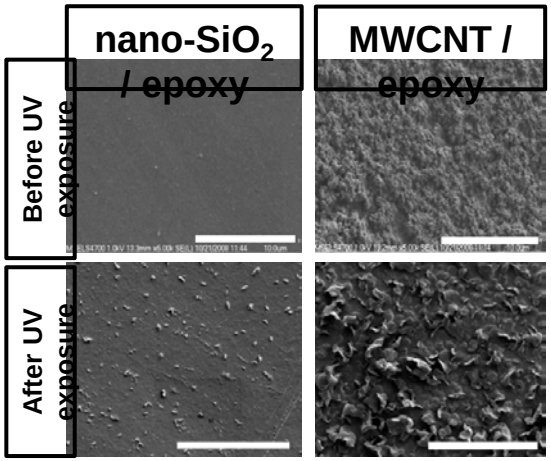
Sotiriou et al., ES: Nano, 2015

Example #3: LCPM Release from Nano-enabled paints/coatings

- **Mechanical wear:**
  - Sanding of acrylic paint with nano-Fe_2O_3 : LCPM (<100 nm) were agglomerates of polymer and Fe_2O_3 ENMs;
    - No free ENMS detected – ***Göhler 2010***
  - Abrasion using Taber Abraser of nano-TiO_2 paint on glass substrate released micrometric and sub-micrometric TiO_2-paint composite particles;
    - No free ENMs detected – ***Golanski 2011***



- **Photochemical degradation:**
  - Progressive UV exposure of epoxy-MWCNT coating destroyed the epoxy matrix and formed a dense network of accumulated MWCNTs on the surface
  - Filler **protected against further degradation** and release of free MWCNTs – ***Nguyen 2011***
  - Progressive UV exposure of epoxy-nano-SiO_2 coating destroyed the epoxy matrix leading to accumulation of SiO_2 nanoparticles on the surface; free SiO_2 nanoparticles passively fell off the exposed surface – ***Nguyen 2012***, ***Nguyen 2011***
  - **Nanofiler properties impact releasability from UV-photodegrated nano-enabled coatings (CNT vs spherical SiO_2 nanoparticle)**

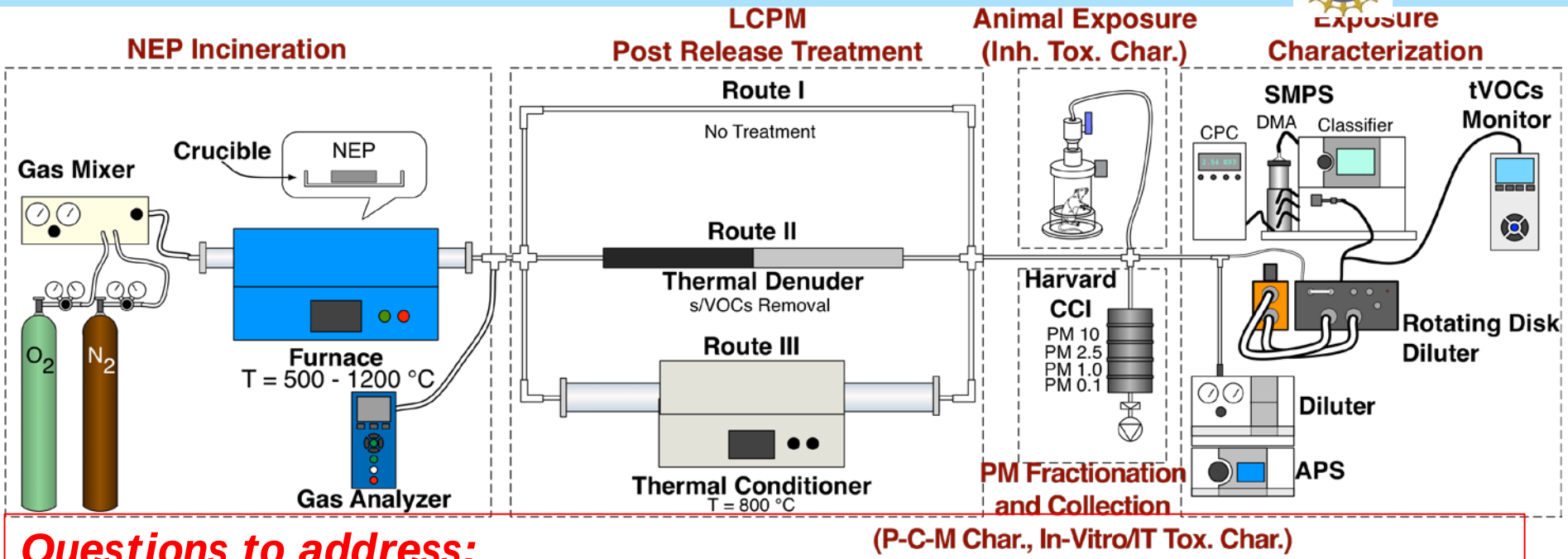


Standardized Accelerated UV aging Method for NEPs

- NIST- integrated **SPHERE exposure chamber (Simulated Photodegradation via High Energy Radiant Exposure)**
- High UV radiant exposure (8400W, 290-400 nm)
- Precisely controlled environment (temperature and humidity)
- Provides continuous and uniform UV exposure to nanocomposites for a desired duration

Nguyen 2011
Chin et al, Review of Scientific Instruments (2004), 75, 4951; Martin and Chin, U.S. Patent 6626053

End of life thermal decomposition/ incineration of NEPs:
Harvard Integrated Exposure Generation System (INEXS)



Questions to address:

- **Nanofiller release in the air during thermal decomposition/incineration ?**
- **Assess the link between host matrix, nanofiller properties , TD conditions and LCPM properties**
- **Fate and transport of by products in env media?**
- **Toxicological characterization of released LCPM ?**
- ~~**Is there a "nanofiller specific toxicological effect"**~~
- **Is there a host polymer tox effect?**

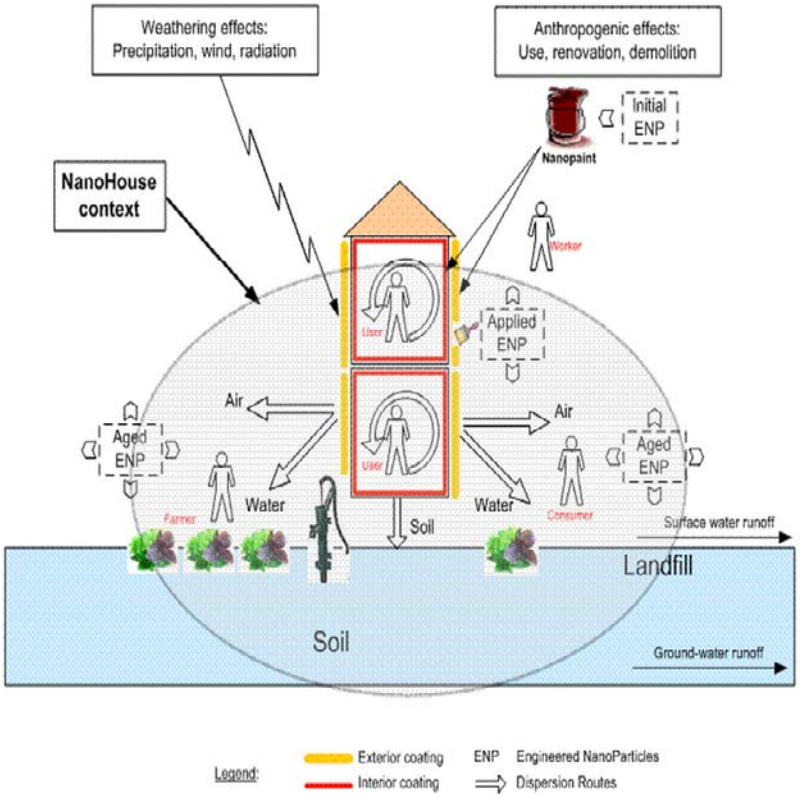
Sotiriou et al, Env. Sci.: Nano,,2015

Current European Union FP7 Projects on Nano Release

- **NANOPOLYTOX** (*http://www.nanopolytox.eu*):
  - Toxicological impact of nanomaterials derived from processing, weathering and recycling from polymer nanocomposites used in various industrial applications
- **NANOHOUSE**

  (*http://www-nanohouse.cea.fr*):
  - Life Cycle of Nanoparticle-based Products used in House Coating
- **NANEX**

  (*http://nanex-project.eu*):
  - Development of Exposure Scenarios for Manufactured Nanomaterials



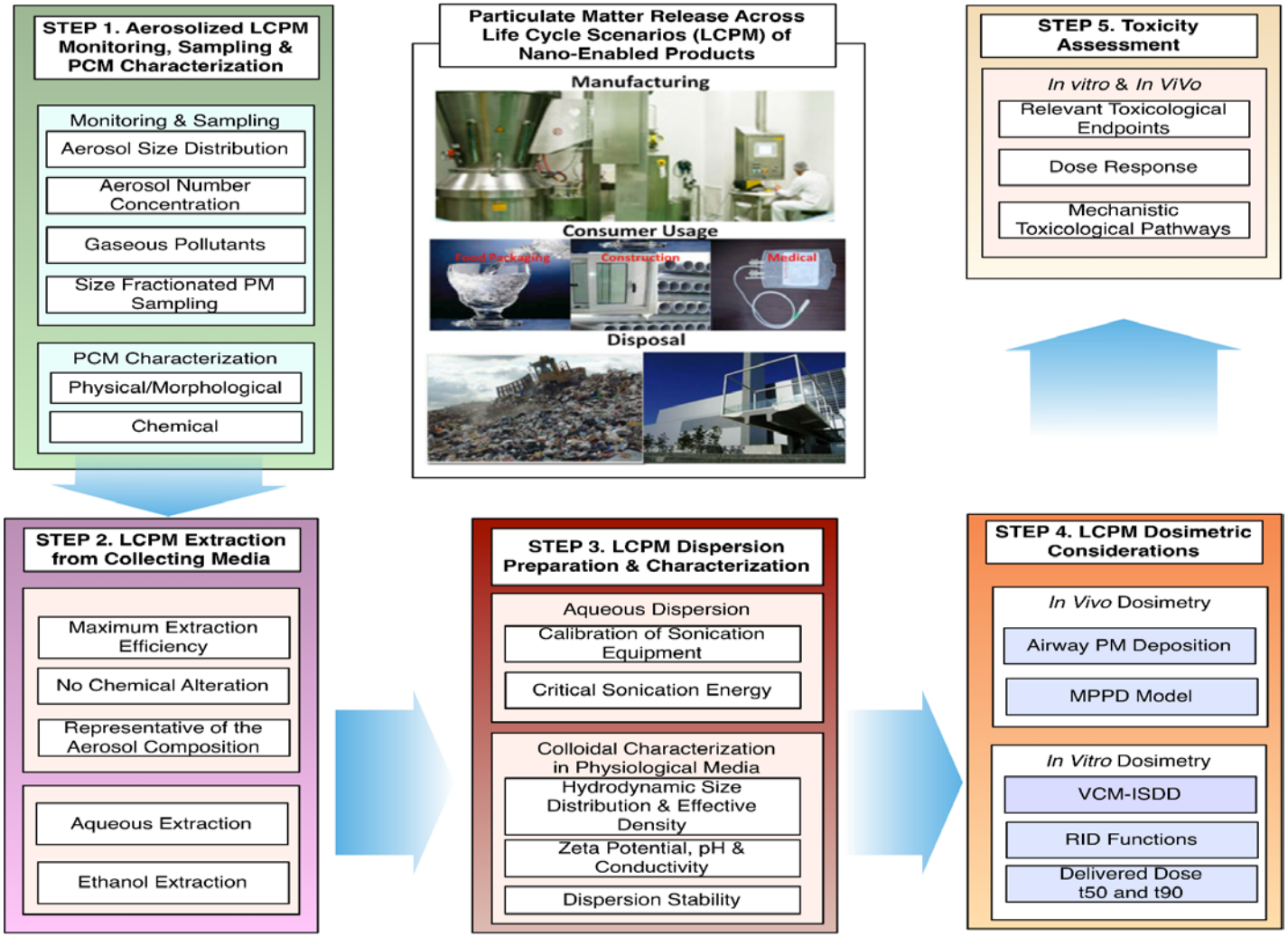
Source: http://www-nanohouse.cea.fr/

Linking LC specific exposures to biological impact:
Emerging integrated methods at the interface of exposure science and toxicology

Linking "real world" LCPM exposures to toxicology and adverse health effects

- It would require new integrated methodologies across the **exposure-toxicology-disease continuum**.
  - Development of **standardized methodologies** which will enable generation of "real world exposures" of LCPM for Families of NEPs (thermoplastics, coatings, etc)
    - Such integrated exposure platforms should be also suitable for pcm and tox characterization.
  - Development and validation of multi-tier toxicological platforms suitable for LCPM exposures.
    - In-vitro cellular assays used in pristine ENM tox assessment are not necessarily suitable to address complexities of LCPM exposures and will require modifications
- **Challenges:** Apportionment of potential tox effects associated with a multi-pollutant mixture, define the nano nanofiller effect; synergistic effects with gaseous co-pollutants.
- It would take time and $$ to develop methodologies across the exposure-disease continuum
- **Ambient PM research to the rescue :** Utilize the knowledge and tools developed for ambient PM toxicological research

Linking LCPM exposures to toxicology: An integrated methodology for Particle Sampling, Extraction, Dispersion and Dosing (SEDD)[1]



Pal et al., Tox. Sci.,,2015

OXFORD

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www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 2015, 1–13

doi: 10.1093/toxsci/kfv095

Advance Access Publication Date: May 20, 2015

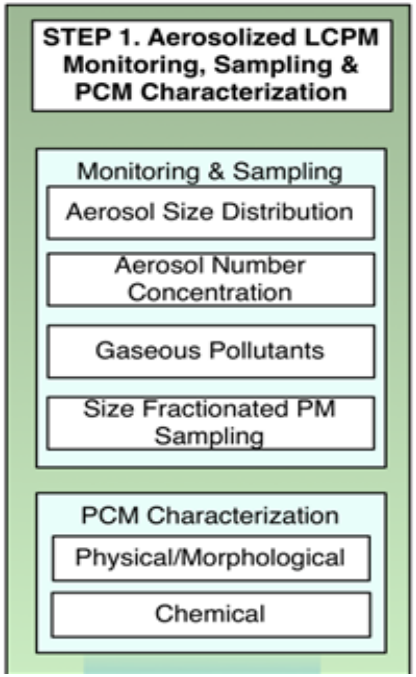
Linking Exposures of Particles Released From Nano-Enabled Products to Toxicology: An Integrated Methodology for Particle Sampling, Extraction, Dispersion, and Dosing

Anoop K. Pal[*,1], Christa Y. Watson[*,1], Sandra V. Pirela[*], Dilpreet Singh[*], Marie-Cecile G. Chalbot[†], Ilias Kavouras[†], and Philip Demokritou[*,2]

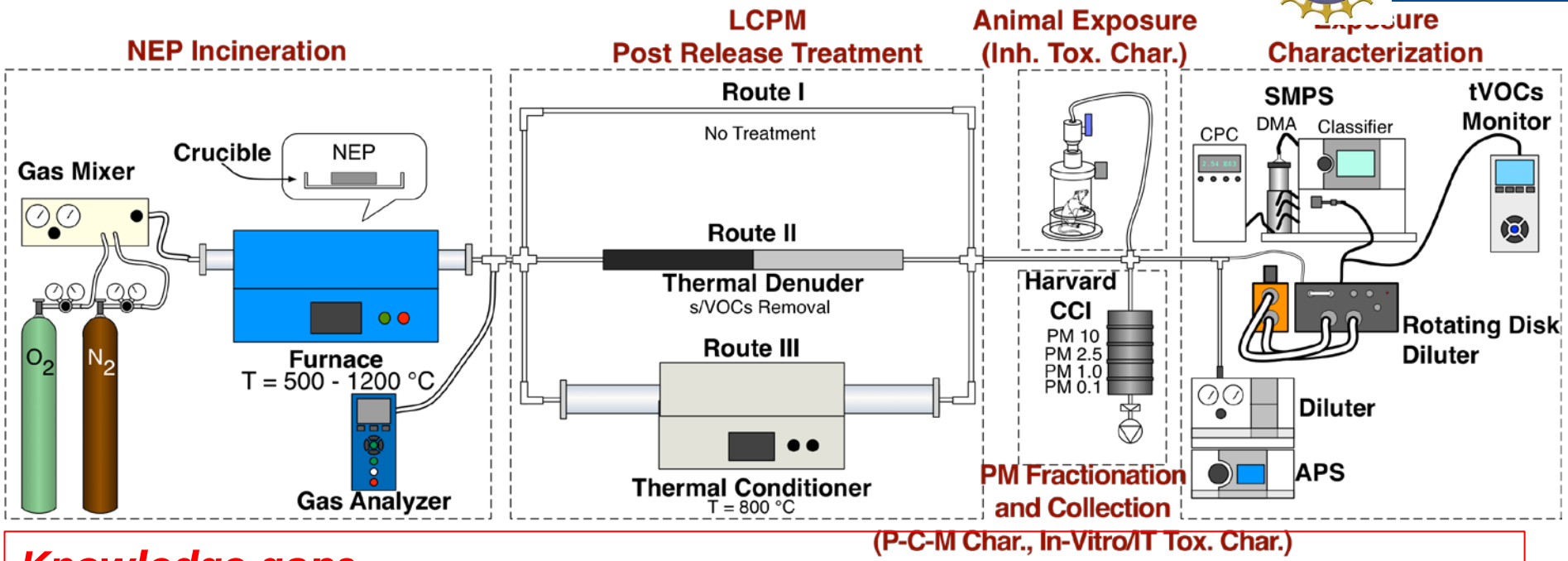
STEP 1: Development of LC specific exposure generation platforms suitable for p-c-m and toxicological characterization of LCPM

FEATURES:

- Emulate real world, LC specific exposure scenarios
- **Challenge:** Standardization and reproducibility
- Exposure generation platforms to:
  - Include both real time and time integrated PM monitoring/sampling systems for p-c-m characterization of LCPM
  - Real time monitoring/characterization of potential gaseous co-pollutants
  - Enable **size fractionated LCPM sampling** for in-vitro and in-vivo instillation tox. studies
  - Suitable for animal in-vivo studies



End of life thermal decomposition/ incineration of NEPs:
Harvard Integrated Exposure Generation System (INEXS)

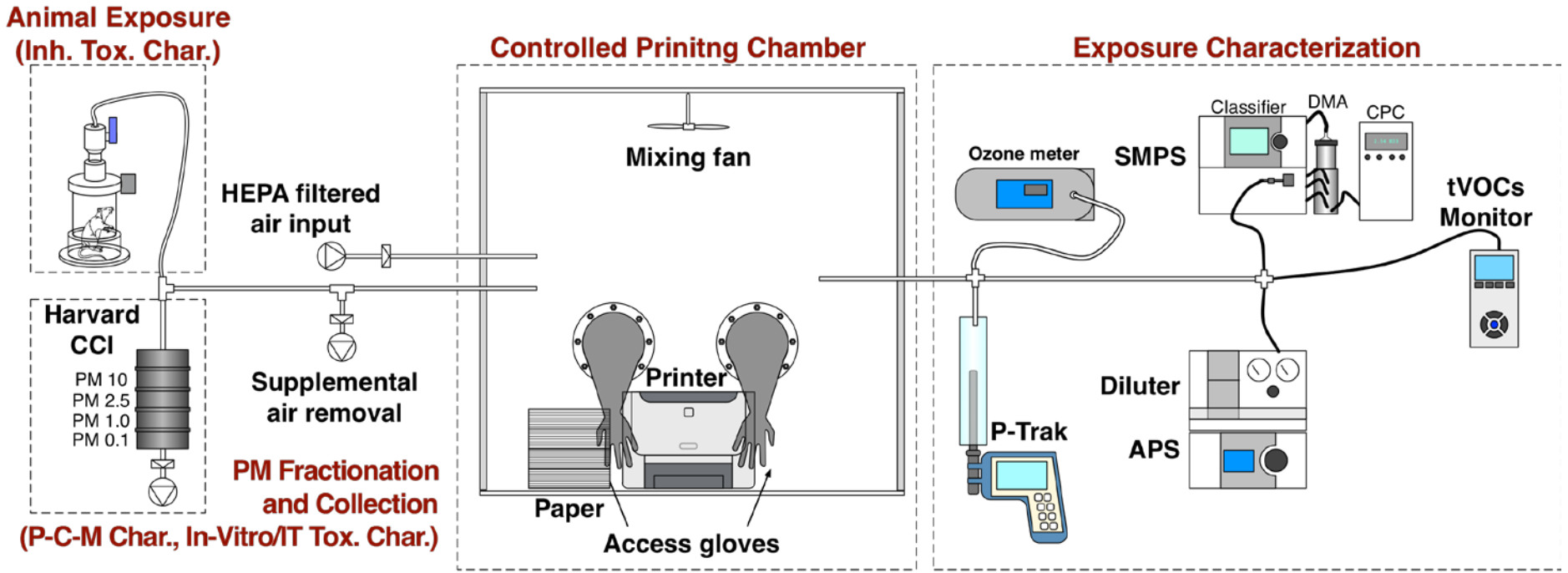


Knowledge gaps

- Nanofiller release in the air during thermal decomposition/incineration ?
- Assess the link between host matrix, nanofiller properties , TD conditions and LCPM properties
- Fate and transport of by products in env media?
- Toxicological characterization of released LCPM ?
- Is there a "nanofiller specific toxicological effect"?

Sotiriou et al, Env. Sci.: Nano.,2015

Example 2: Development of Printer Exposure Generation System (PEGS):
Tox implications from ENMs released during printing from nano-enabled toners

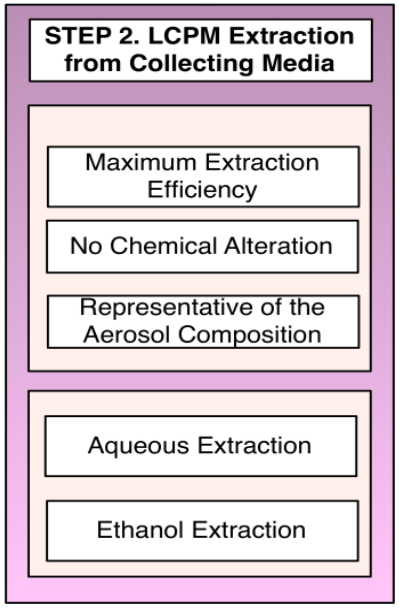


Features:

- Uninterrupted operation
- Real time LCPM and gaseous co-pollutant monitoring
- Size fractionated LCPM sampling for pcm and in-vitro/IT tox characterization
- Animal inhalation tox studies
- Simulation of different exposure scenarios

Pirela et al., *Inhalation Toxicology* 2014

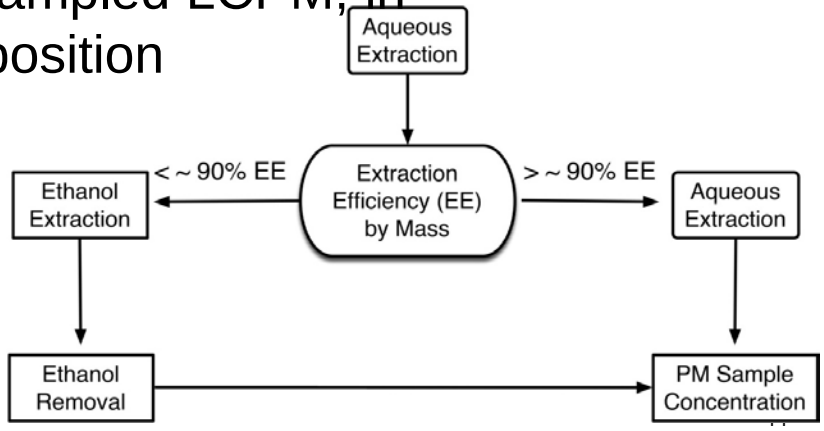
STEP 2: Size fractionated LCPM extraction from collection Media



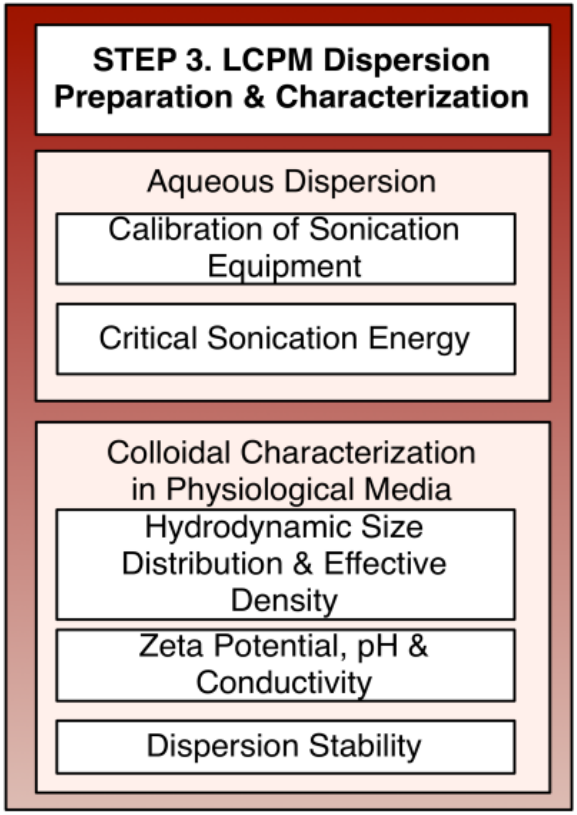
Challenge: Efficient extraction from collection media of sampled LCPM with minimum physico-chemical modifications

SEDD methodology ensures:

- Maximum recovery of collected particle mass using aqueous or ethanol extraction protocol
- Minimum contamination by the components of collection substrate itself
- Extracted sample representative of the sampled LCPM, in terms of size and organic/inorganic composition



STEP 3: LCPM Dispersion preparation and characterization



SEDD approach:

- **Create stable LCPM suspensions with minimal agglomeration for in-vitro tox studies.**
  - **Particle sonication - delivered** critical sonication energy
  - Colloidal stabilization with serum proteins
- **Performing colloidal characterization of suspensions to include measurements:**
  - Size distribution, zeta potential, pH & conductivity
  - Effective density – defines bioactivity and F&T

Selected publications on colloidal preparation and characterization

Nanotoxicology, June 2013; 7(4):417–431
© 2013 Informa UK, Ltd.
ISSN: 1743-5390 print / 1743-5404 online
DOI: 10.3109/17435390.2012.666576

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healthcare

Interactions of engineered nanomaterials in physiological media and implications for *in vitro* dosimetry

Joel Cohen, Glen DeLoid, Georgios Pyrgiotakis, & Philip Demokritou

Department of Environmental Health, Center for Nanotechnology and Nanotoxicology, Harvard School of Public Health, Boston, MA, USA

Cohen *et al. Particle and Fibre Toxicology* 2014, **11**:20
http://www.particleandfibretoxicology.com/content/11/1/20

pft PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

An integrated approach for the in vitro dosimetry of engineered nanomaterials

Joel M Cohen[1], Justin G Teeguarden[2] and Philip Demokritou[1*]

nature COMMUNICATIONS

ARTICLE

Received 15 Jul 2013 | Accepted 26 Feb 2014 | Published 28 Mar 2014

DOI: 10.1038/ncomms4514

Estimating the effective density of engineered nanomaterials for *in vitro* dosimetry

Glen DeLoid[1,*], Joel M. Cohen[1,*], Tom Darrah[2], Raymond Derk[3], Liying Rojanasakul[3], Georgios Pyrgiotakis[1], Wendel Wohlleben[4] & Philip Demokritou[1,*]

http://informahealthcare.com/nan
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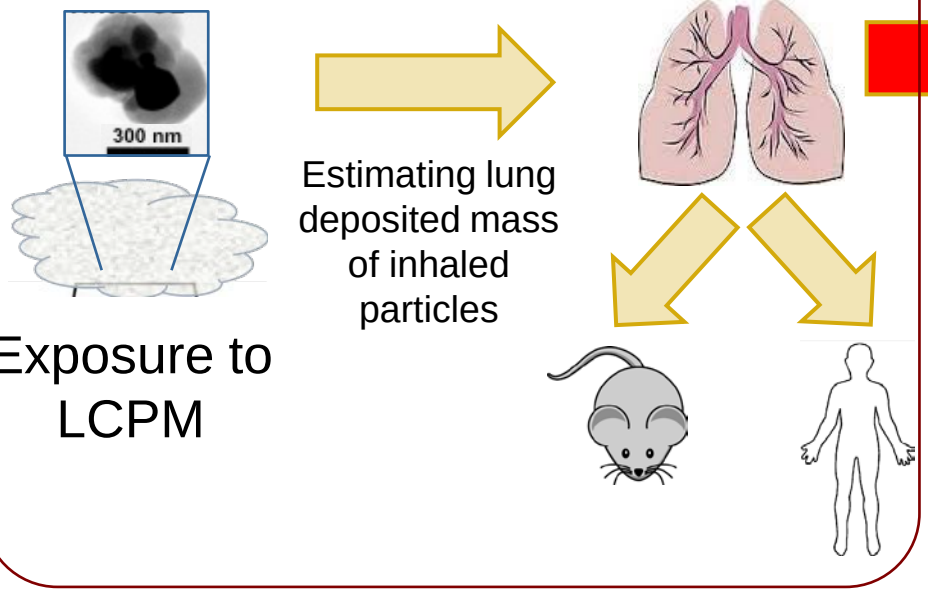
ORIGINAL ARTICLE

Implications of *in vitro* dosimetry on toxicological ranking of low aspect ratio engineered nanomaterials

Anoop K. Pal[1,3], Dhimiter Bello[2,3], Joel Cohen[3], and Philip Demokritou[3]

[1]*Biomedical Engineering and Biotechnology Program, University of Massachusetts, Lowell, MA, USA,* [2]*Department of Work Environment, College of Health Sciences, University of Massachusetts, Lowell, MA, USA, and* [3]*Center for Nanotechnology and Nanotoxicology, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA*

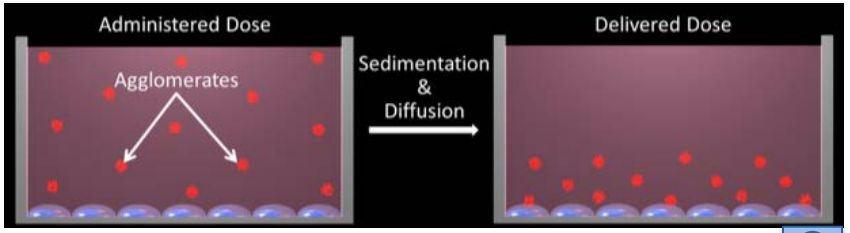
STEP 4: Emerging tools and approaches for bridging the gap between exposure and in-vitro/in-vivo dosimetry of ENMs



Deposited dose *in vitro*

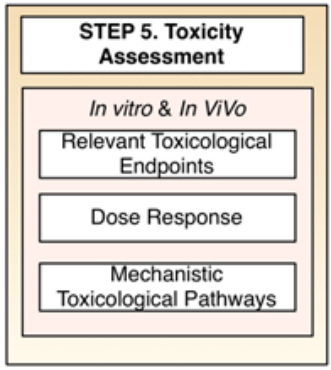
Media + Cell line + Particle

Estimating In-vitro Administered dose using the Harvard In-vitro dosimetric platform[2-4]



[1] Angilvel, 1995 | [2] Demokritou et al., 2013 | [3] Cohen et al., 2014 | [4] DeLoid et al., 2014

STEP 5: LCPM Toxicological assessment



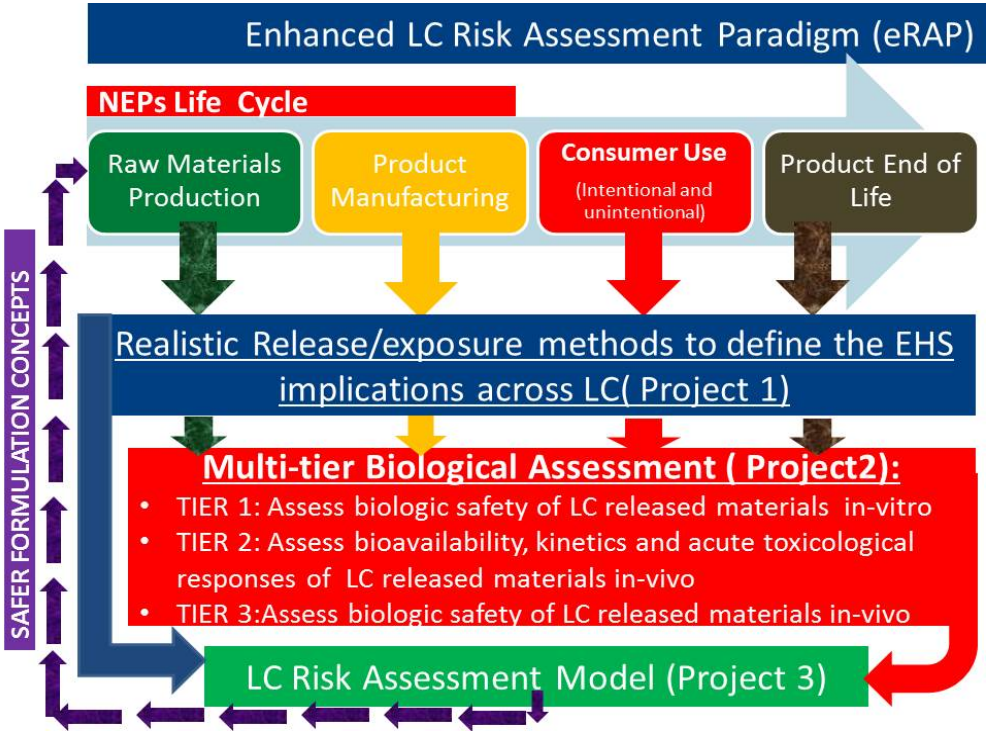
- **Multi-tier tox screening** using both cellular and animal models
  - In-vitro: Assess dose/response relationships, understand the mechanism of bioactivity (cytoxicity, mitochondrial activity, ROS production, DNA damage, cell function, epigenetic modifications, gene expression, *etc)*
  - Only the most bioactive LCPM are evaluated using in-vivo animal models

Challenges:

- **In-vivo LCPM tox** screening for all LC specific scenarios could be laborious and costly
- In-vitro assays for pristine ENMs might not be adequate for LCPM tox screening
- **Apportionment** of potential nanofiller tox effect:. Multipollutant models are needed
- **Synergistic effects** from gaseous co-pollutants

Pal *et al*., *Toxicological Sciences* 2015

Where should we go from here? Enhanced LC Risk Assessment Paradigm (eRAP):



- Nano-Risk: Expand RAP beyond “raw ENMs” and occupational exposures to include LC implications
- Need to develop standardized methods to assess Release and Exposures across LC of NEPs
- Need to develop multi-tier toxicological screening tools to link exposures to toxicology
- Develop safer by design approaches to minimize risks

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- Dr. Sotiris Pratsinis, ETH Zurich
- Dr Christa Watson, HSPH
- Sandra Pirella, Doctoral Student, HSPH
- Dr Georgios Pyrgiotakis, HSPH
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- *Dr. Anoop Pal, Umass, Lowell*
- *Dr Wendel Wohlleben, BASF*
- *Dr Justin Teeguarden, PNNL*

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WHERE DISCOVERIES BEGIN

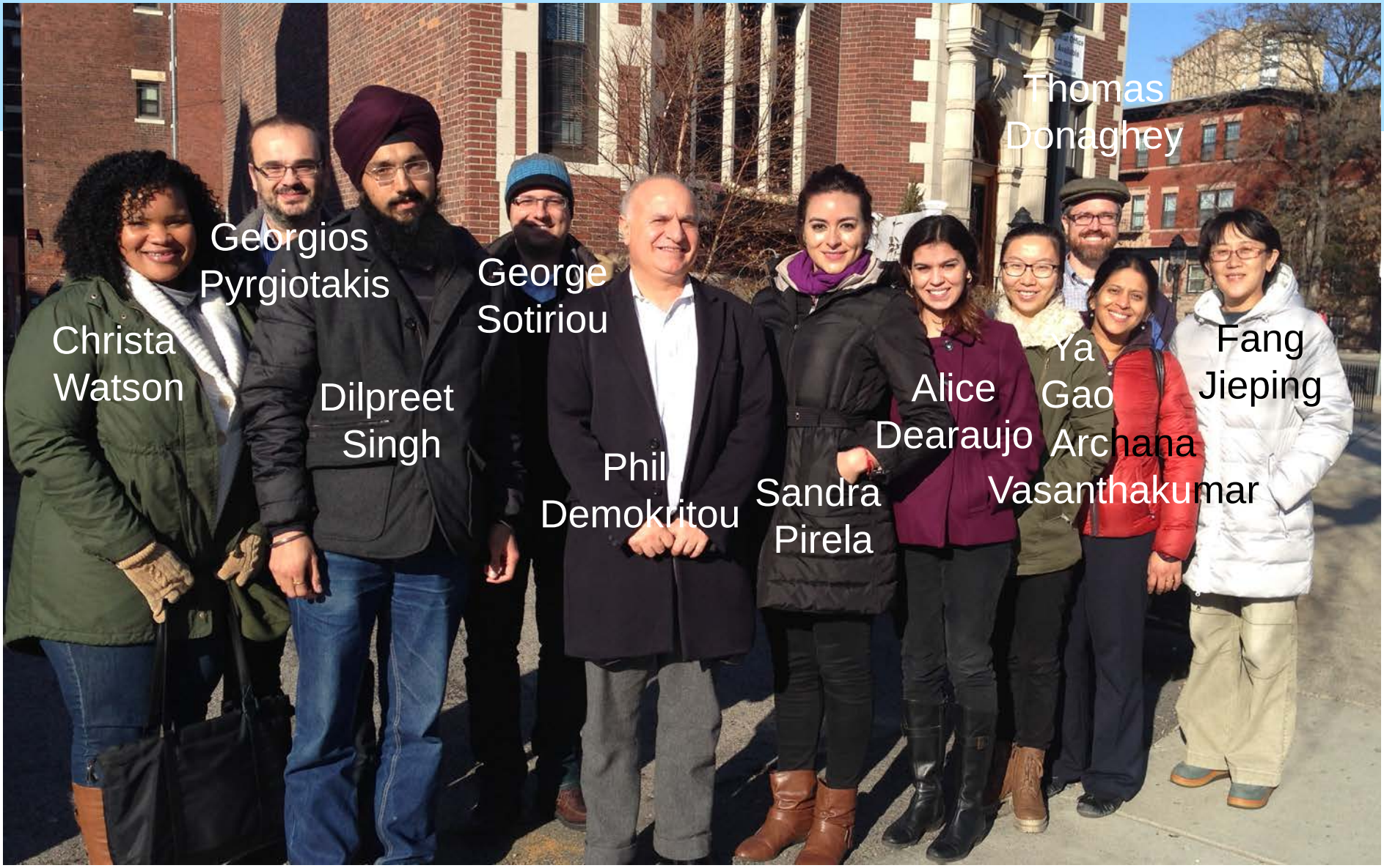
NIEHS
National Institute of Environmental Health Sciences

NIOSH

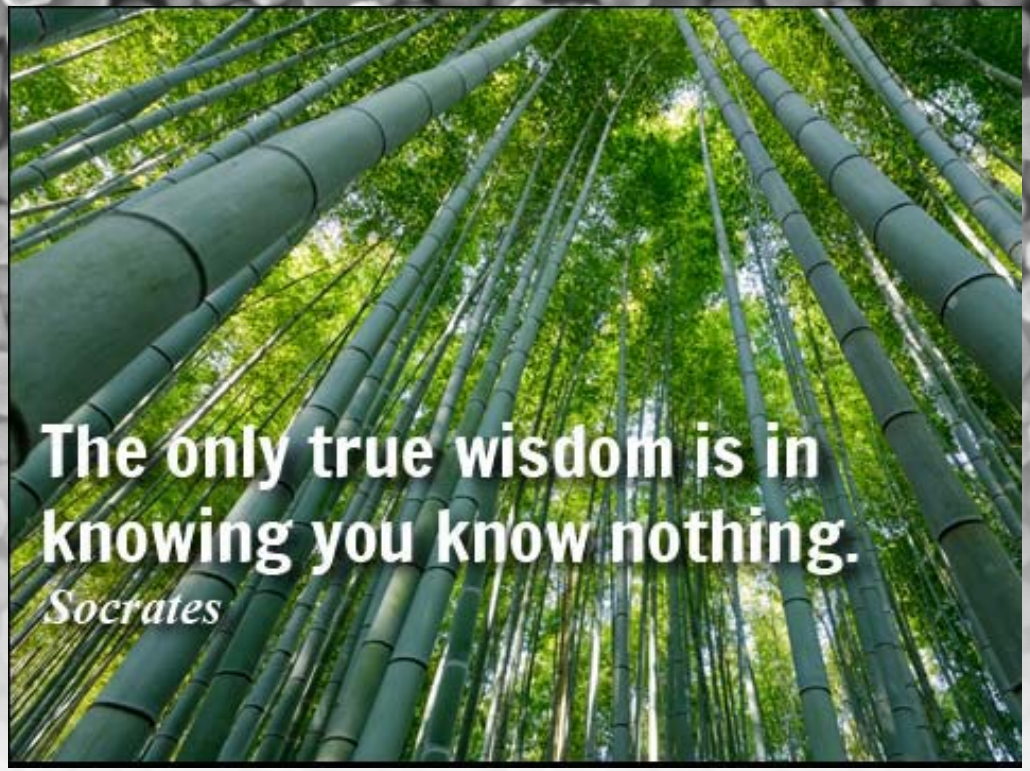
UNITED STATES OF AMERICA
CONSUMER PRODUCT SAFETY COMMISSION

USDA

BASF


Thomas Donaghey
Georgios Pyrgiotakis
George Sotiriou
Christa Watson
Dilpreet Singh
Phil Demokritou
Sandra Pirela
Alice Dearaujo
Ya Gao
Archana Vasanthakumar
Fang Jieping

Questions?



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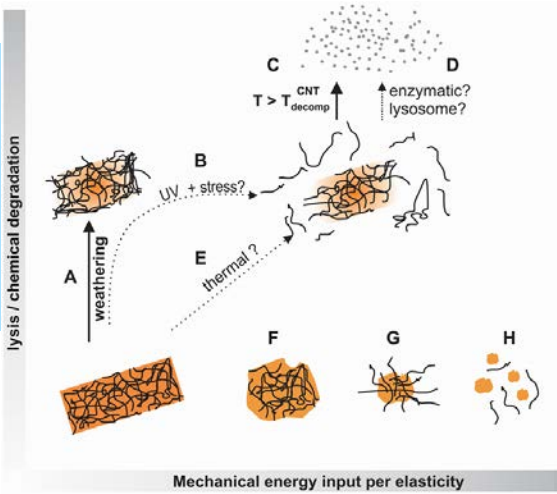


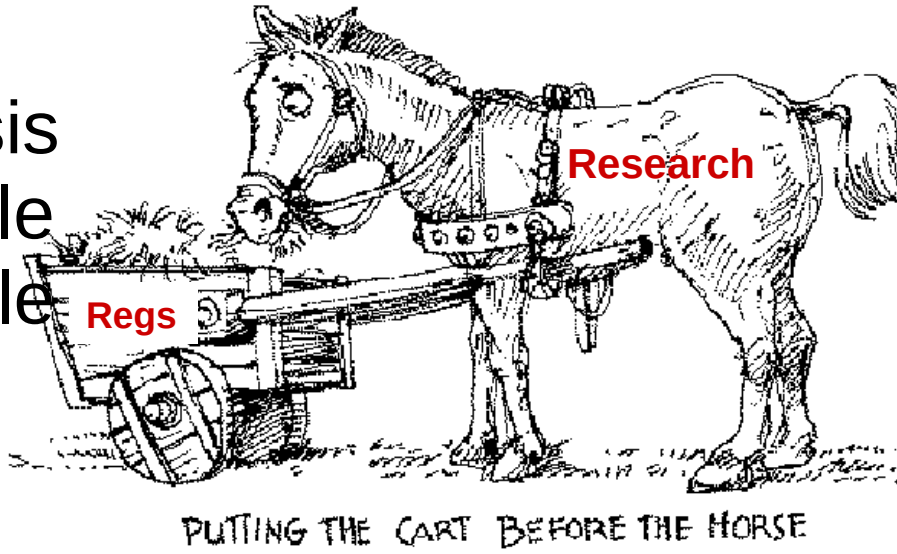
- Focus on release characteristics of **MWCNTs from Polymer Composites**
- **Key Results –**
  - Must re-align the main focus of EHS attention to study of what is released.
  - Virtually all release from composites was dominated by matrix NOT by nanofiller.
  - Need basic methods development to describe quantitatively what is nano of concern in a realistic release.

Major knowledge gap: EXPOSURE data at human population and environmental levels

- Exposure data at human/environmental level are **fragmentary**
- Current exposure data are primarily for occupational settings and associated with **handling/synthesis of pristine ENMs**
- ENM properties change in both **value-chain**, and across the **life cycle** of nano-enabled products
- Assessing potential **ENM release pathways and dynamics** for life cycle scenarios for families of NEPs is at its infancy
- “Real world” exposure and tox data across life cycle of NEPs are fragmentary but are required to **assess Risks beyond occupational settings**.

Learn from other environmental contaminants:
Research first - regulations later

- **Regulations:** Need to be science based
- Do we regulate ENMs based on the tox profile of “raw” materials used in the synthesis of NEPs or “real world” particle exposures across the life cycle of NEPs
- **ENM definitions**: Are NOT science based
- “Size” Vs “behavour” based definition?



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