The utility of Alternative Testing Strategies in Nanotechnology Health and Safety

The California NanoSystems Institute (CNSI)

***188,000 sq. ft. facility** with research labs, technology centers, start-up incubator, administrative support, conferencing and interaction space*

UNIVERSITY OF CALIFORNIA
UC CEIN Center for Environmental Implications of Nanotechnology

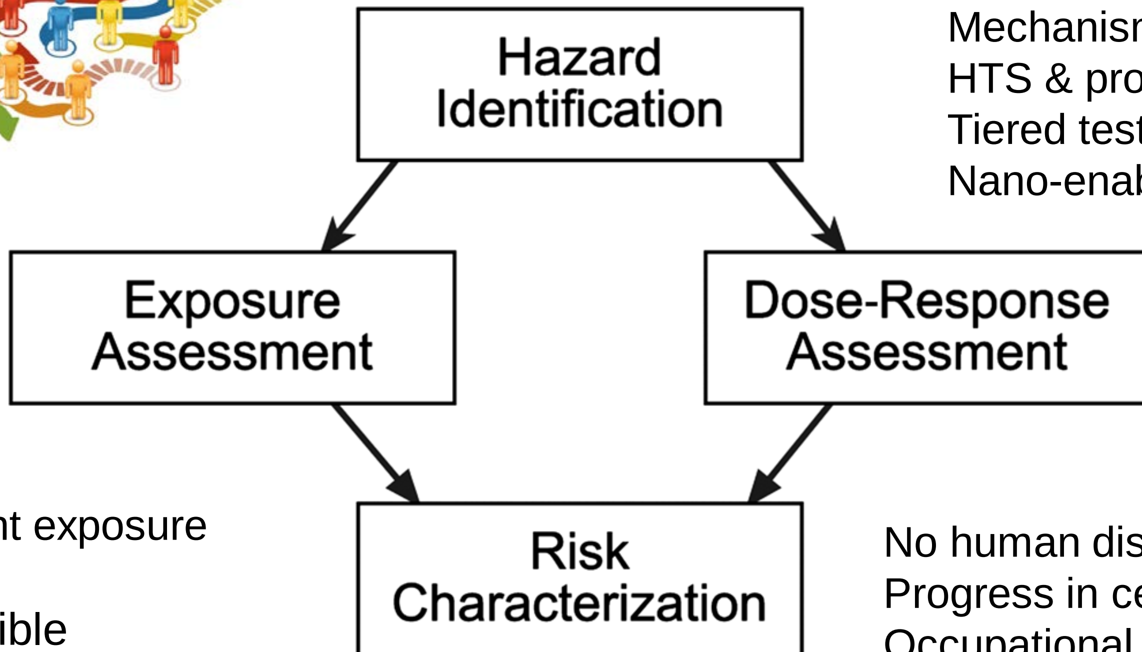
NIEHS
National Institute of Environmental Health Sciences

Nano-Go
NCN-HIR
NHIR

André Nel M.B.,Ch.B; M.D.
Distinguished Professor of Medicine and Chief of NanoMedicine
Associate Director of the California NanoSystems Institute
Director UC Center for the Environmental Implications of Nanotechnology
Associate Editor ACS Nano

Nano EHS

Pristine Materials
New properties
“New chemical substances”
Case-by-case chem tox
Descriptive animal studies
↓
Mechanism-based screening
HTS & profiling of categories
Tiered testing approaches
Nano-enabled & composites



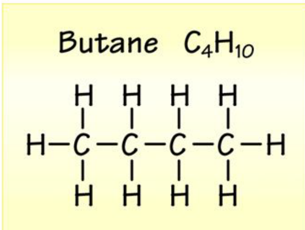
What are relevant exposure conc’s?
Aerosols - possible
Workplace monitoring for inhalants (OELs)
Biological tissue-difficult
Environment - very difficult
Instrumentation needs

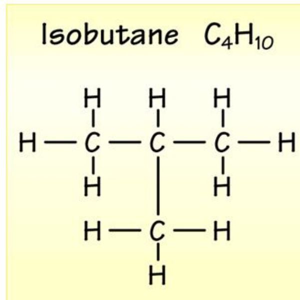
Little quantitative RA
Some qualitative RA
OELS
Tiered risk assessment
Integrated approaches to decision making (IATA)

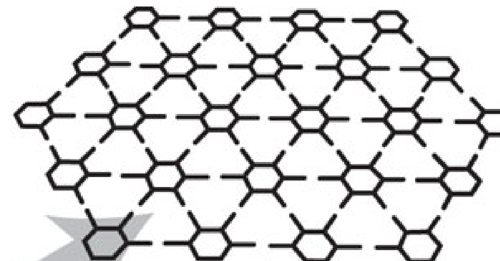
No human disease models
Progress in cellular dose calc
Occupational data - OELS
Benchmark materials
In vitro/in vivo extrapolations

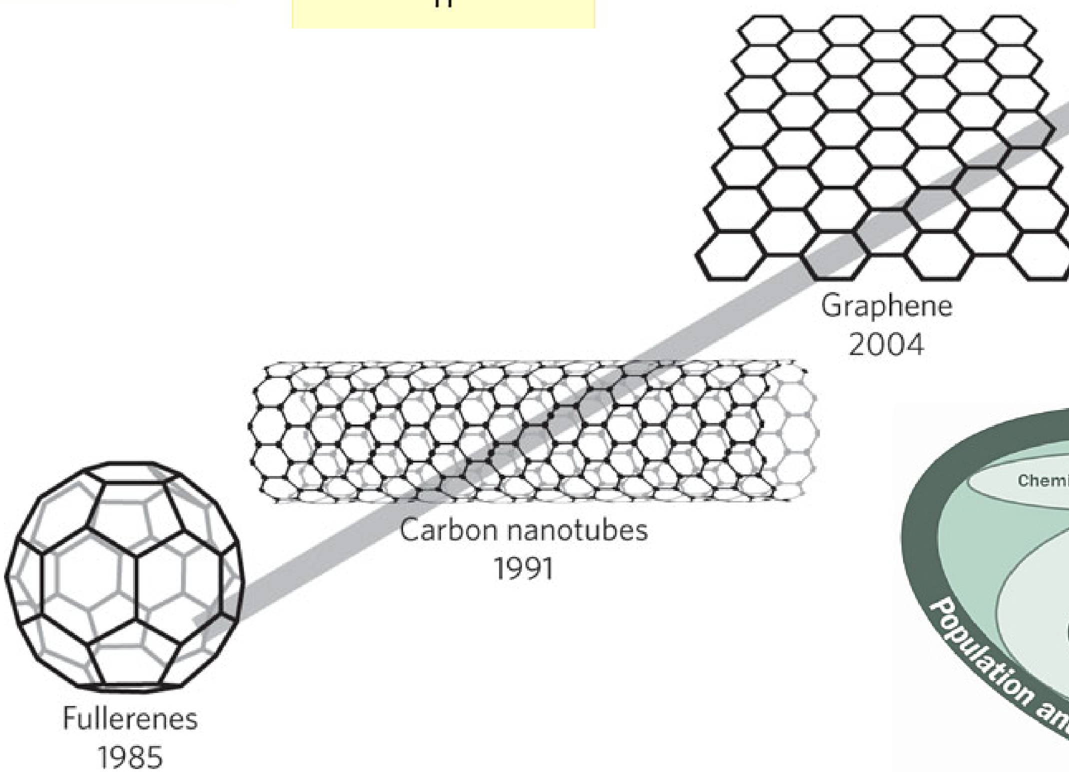
Chemical Toxicity Principles not Optimal for Molecular Initiation or Triggering Events but useful for AOP assessment

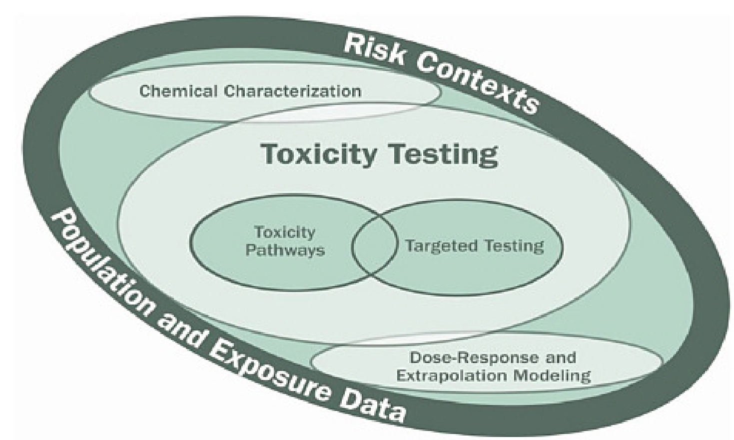
Carbon as an example…..











There are Unique Nanoscale Characteristics that Contribute in a Unique Way to Trigger Molecular Injury

Properties

Intrinsic — Material as acquired or synthesized

- *Chemical composition*
- *Crystal structure/crystallinity*
- *Particle size/size distribution*
- *Purity*
- *Shape*
- *Surface area and rugosity*
- *Surface functions/coatings*

Extrinsic — Altered properties in biological media

- *Charge: EFM and ζ-potential*
- *Corona*
- *Dissolution*
- *Surface reconstruction*
- *Sorption*

Emergent properties — Tox- SARs proximately associated with injury

- High aspect ratio injury to lysosomes
  (CNTs, CeO_2 nanorods)
- Bandgap in semiconductor NPs
- Silica siloxane rings and silanols
- Reactive surface functionalities
  (hydroxyls, charged groups, surface defects, carbonyl radicals, rare earth phosphate complexation)

Nel et al. Science 2006
Nel et al. Nature Materials. 2009

The Frank R. Lautenberg Chemical Safety for the 21st Century Act (H.R. 2576), a.k.a. the TSCA Reform Bill

- Mandates, for the 1st time, the safety evaluation of all existing chemicals in commerce and industry, starting with those most likely to cause risks
- Evaluate and prioritizes new and existing chemicals against new science & risk-based safety standards, including considerations for vulnerable populations
- Require affirmative chemical/physchem data collection to support safety evaluation
- Clear and enforceable deadlines and timely action on identified risks
- Increase transparency of chemical information by limiting unwarranted claims of confidentiality, allowing appropriate sharing of confidential information
- Providing a source of funding for EPA to carry out these significant new responsibilities

June 22nd, 2016

TSCA Reform Provisions for Alternative Test Strategies

" (3D) prior to adopting a requirement for testing using vertebrate animals, the Administrator is required to take into consideration, as appropriate and to the extent practicable, reasonably available:

(i) toxicity information;

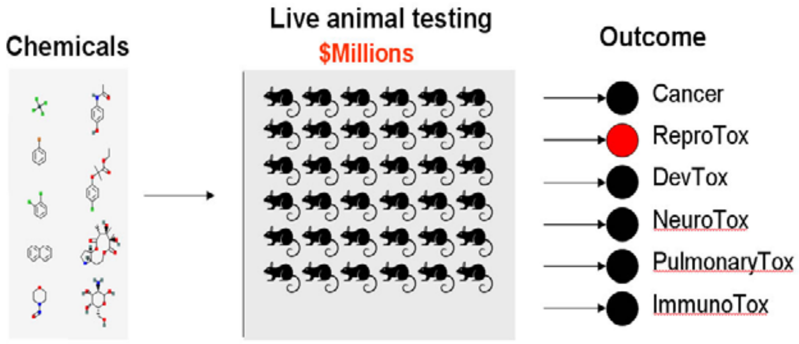
(ii) computational toxicology and bioinformatics;

(iii) high-throughput screening methods and the prediction models of those methods; and

(iv) scientifically reliable and relevant alternatives to tests on animals that would provide equivalent information."

"(4) TIERED TESTING.-

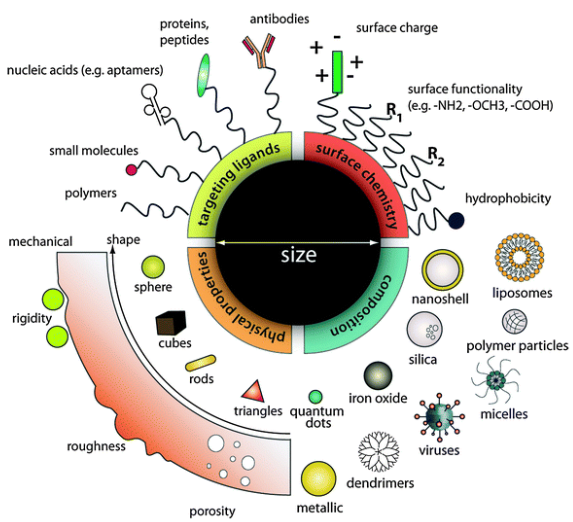
(A) IN GENERAL.-….the Administrator shall employ a tiered screening and testing process, under which the results of screening-level tests or assessments of available information inform the decision as to whether 1 or more additional tests are necessary."

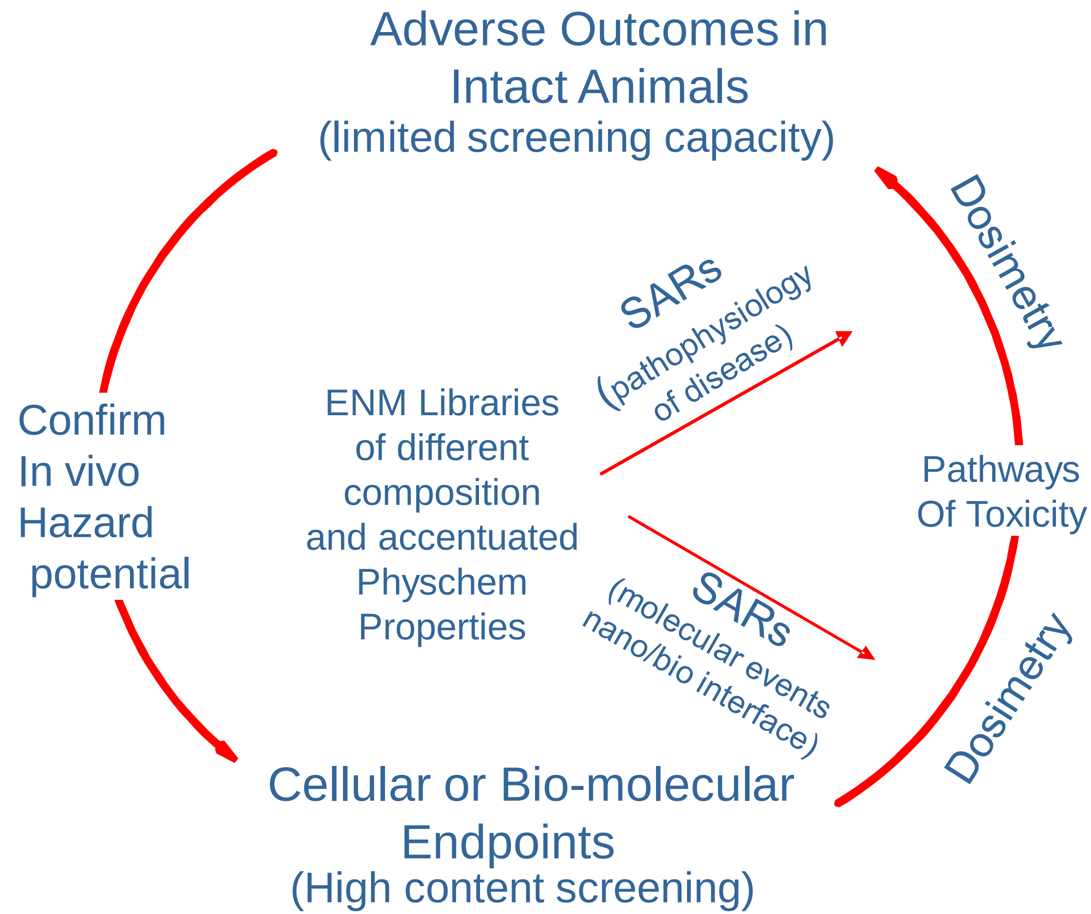


UC CEIN Predictive Toxicological Modeling

1000's of new materials

Physicochemical complexity





Nel et al. Nature Material, 2009
Xia et al, ACS Nano, 2008
Xia et al. ACS Nano. 2011

George et al. ACS Nano. 2010
George et al. ACS Nano. 2011
George et al JACS 2011

Lin et al. ACS Nano. 2011
Xia et al ACS Nano. 2009
Zhang et al ACS Nano 2011

Wang et al. ACS Nano. 2010
Wang et al ACS Nano. 2011

Organization for Predictive Toxicological Research UC CEIN

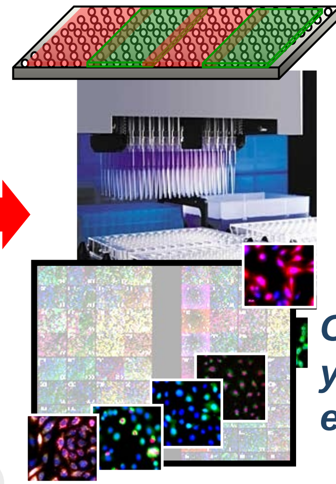
Nanomaterial libraries

Compositional
Metal Oxides
Metals
CNTs

Property accentuation
Size, Shape, AR
Dissolution
Band gap

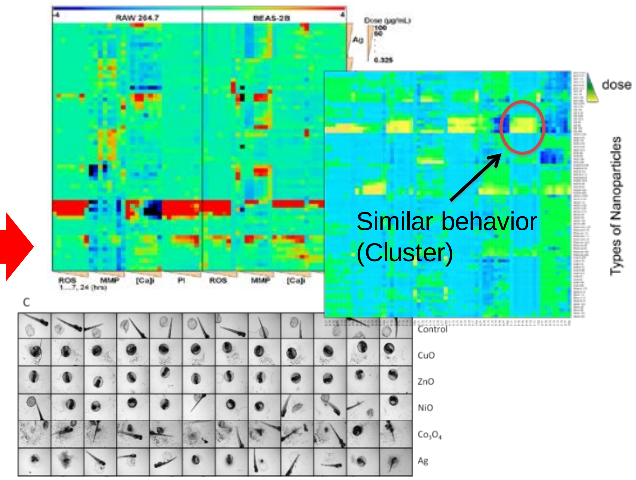
Commercial nanoproducts

High throughput screening



Cells, bacteria, yeasts, zebrafish embryos

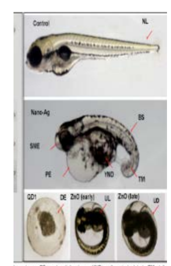
In silico decisions, in vitro ranking

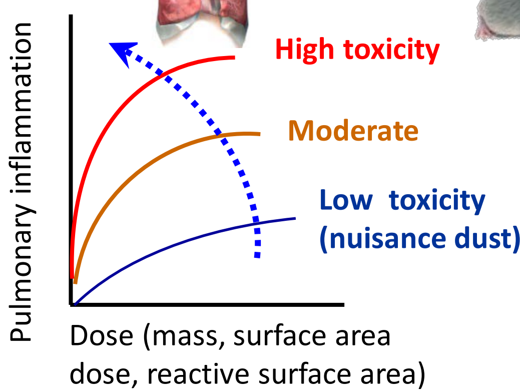


Prioritize
Compare
Speed up

Validate
Dosimetry
Refine

Organism
Animal testing





Risk Identification

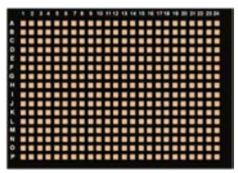
and decision making to

- Reduce risk
- Influence governance
- Dosimetry calculations
- Safer design

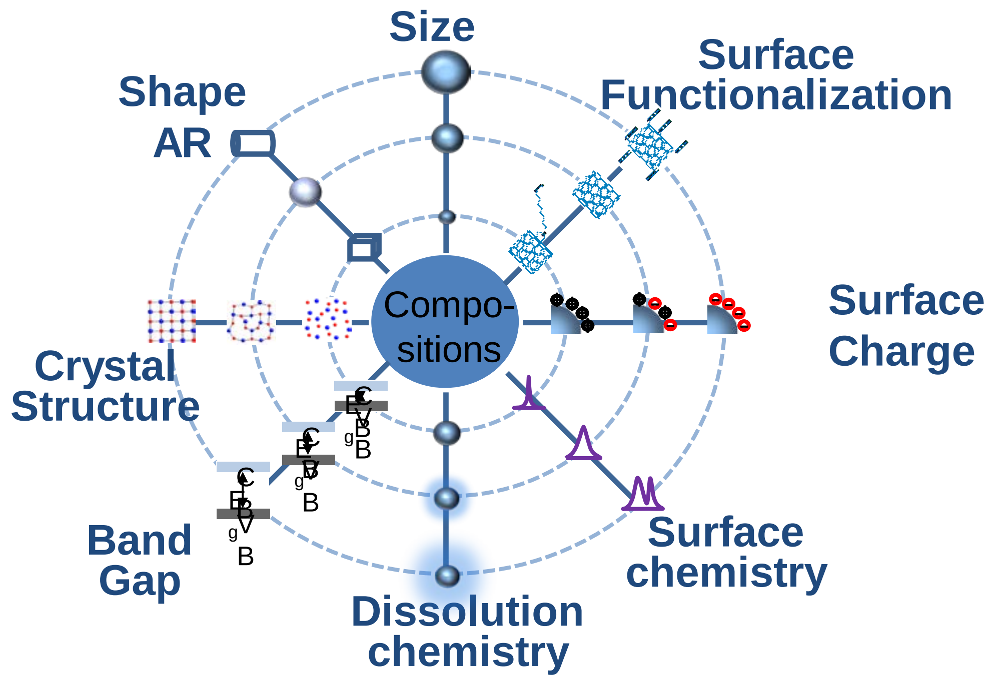
In vivo hazard ranking

Tools to establish Predictive Models

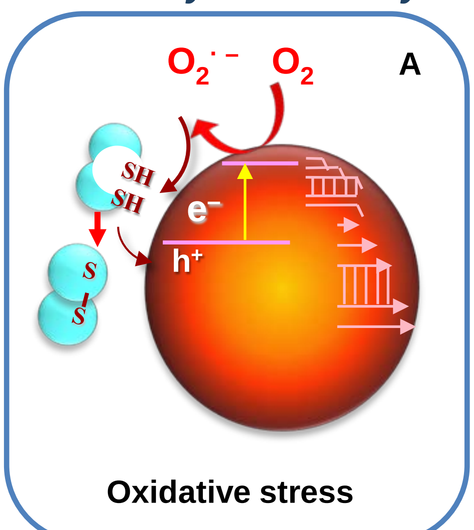
High Throughput Screening



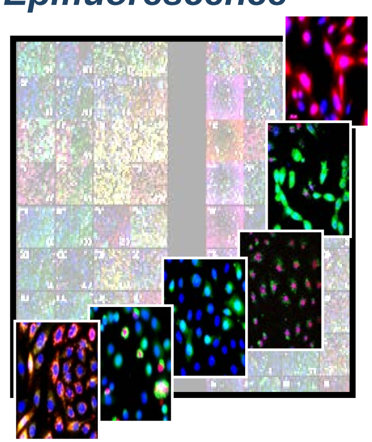
Composition and Combinatorial ENM Libraries



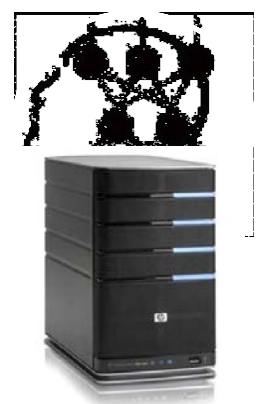
Pathways of Toxicity



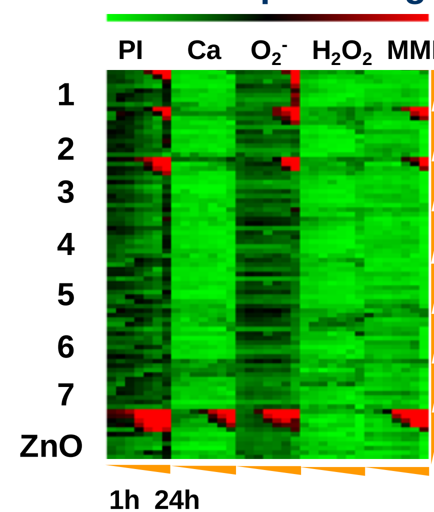
Epifluorescence



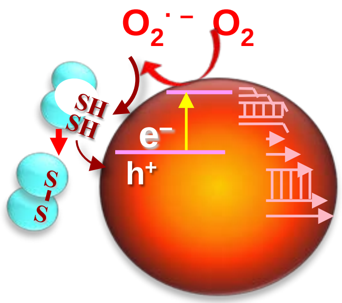
Computational analysis



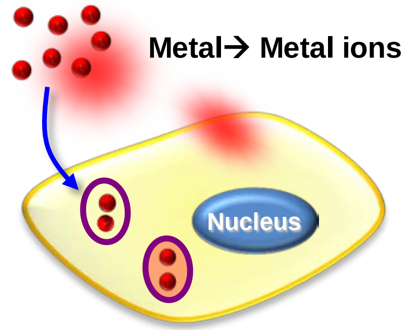
Heat map ranking



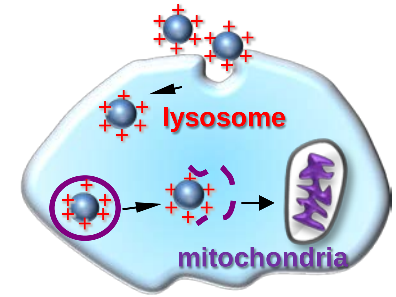
From Pathways of Toxicity to Adverse Outcome Pathways



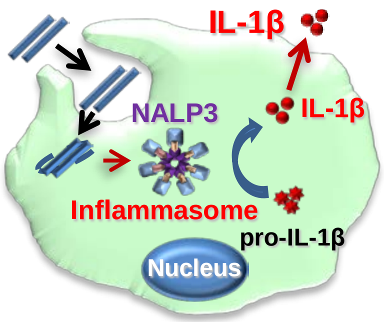
Redox activity and ROS
e.g., TiO_2, CuO, CoO



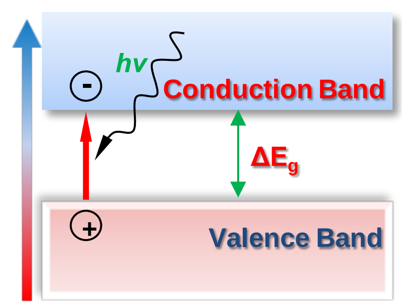
Dissolution, shedding toxic Ions, *e.g.*, ZnO, CuO



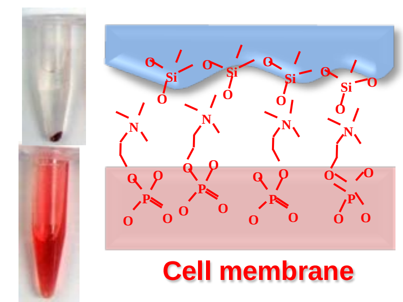
Cationic toxicity, *e.g.*, cationic NH2-polystyrene, PEI-Si



Inflammasome activation
e.g., High aspect ratios



Bandgap & Photoactivation
e.g., Transition MOX's



Membrane Lysis
e.g., fumed SiO_2,

Adverse Outcome Pathway

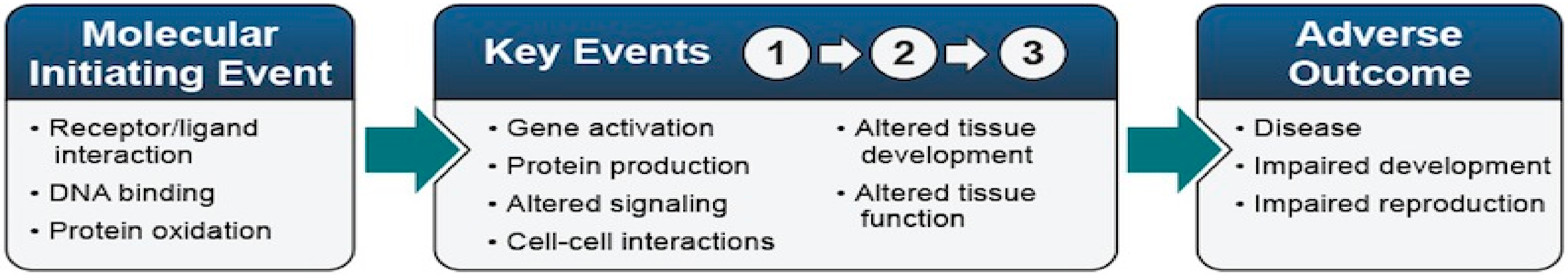
Nel et al. Nature Material, 2009
Xia et al, ACS Nano, 2008
Xia et al. ACS Nano. 2011

George et al. ACS Nano. 2010
George et al. ACS Nano. 2011
George et al JACS 2011

Lin et al. ACS Nano. 2011
Xia et al ACS Nano. 2009
Zhang et al ACS Nano 2011

Wang et al. ACS Nano. 2010
Wang et al ACS Nano. 2011

Adverse Outcome Pathways: A Framework for Organizing Information for Predictive Toxicological Modeling

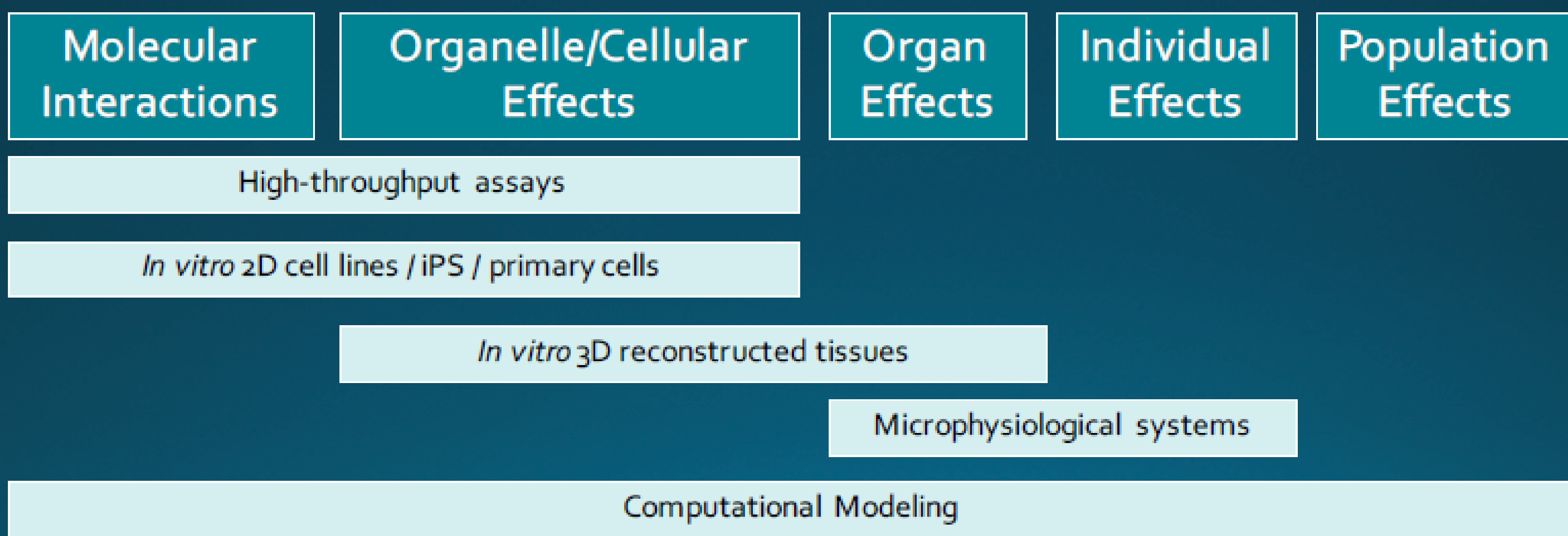


Combines information from multiple fields of inquiry to illuminate knowledge of biological pathways, highlight species differences or similarities, identify research needs, and support regulatory decisions.

Adapted from Kristie Sullivan, Physicians Committee for Responsible Medicine

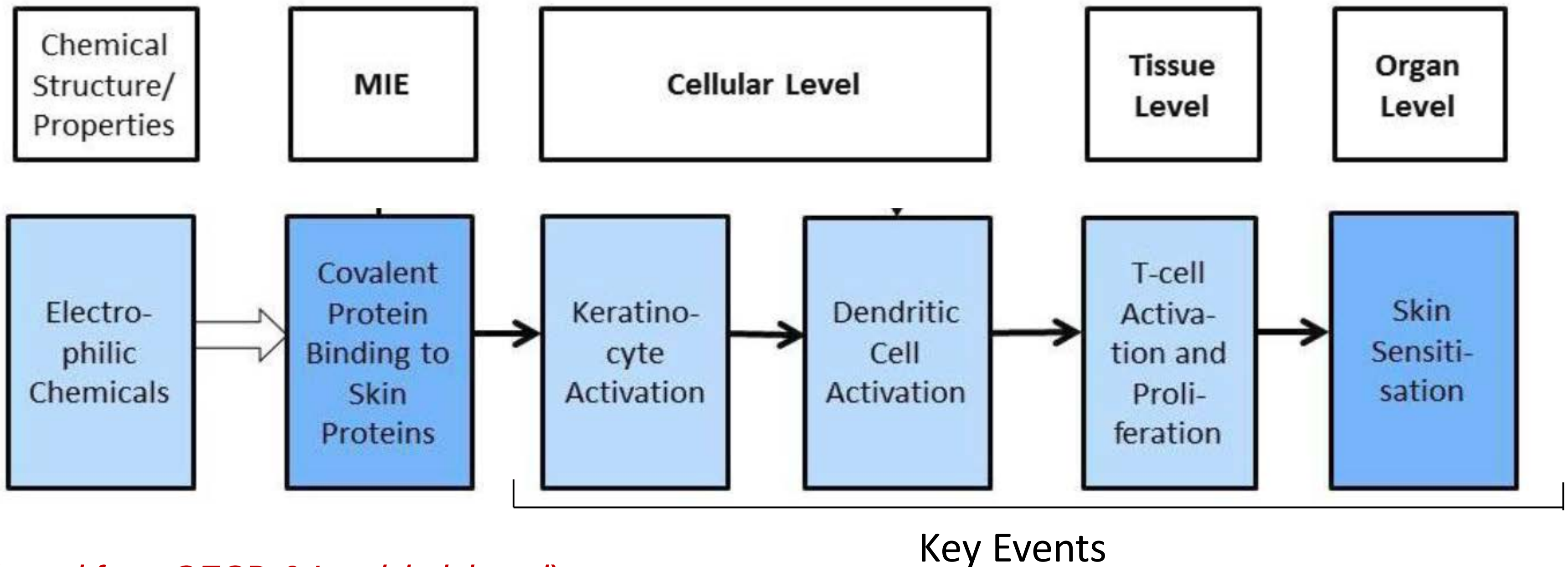
Molecular Interactions	Organelle/Cellular Effects	Organ Effects	Individual Effects	Population Effects
• Receptor interaction • Covalent binding	• Gene activation • Oxidative stress • Immune cell activation	• Tissue pro-liferation • Altered function	• Altered repro-duction • Disease • Lethality	• Extinction

AOPs link Molecular and Mechanistic events to Integrated Approaches to Testing and Assessment (IATA) that can be supported by High Throughput Screening and Computational Modeling for Regulatory Purposes



Adapted from Kristie Sullivan, Physicians Committee for Responsible Medicine

Flow diagram of the steps in the AOP pathway, MIE and Key Events associated with Skin Sensitization



(adapted from OECD & Landsiedel et al)

<u>Example Cosmetic Chemical Test Series</u>:

- Non-cosmetic chemicals
- Actives
- Dyes
- Fragrances
- Preservatives
- Surfactants

cosmetic chemicals

KE1: Direct Peptide Reactivity Assay

KE2: KeratinoSens™ Oxidative Stress Assay

No

Yes

Classify: 2 concordant results from 2 tests

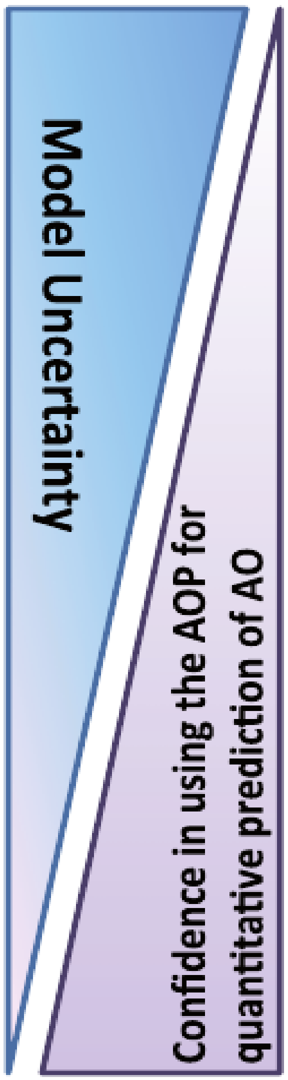
KE3: Antigen Presenting Cell Activation Assay

Yes

Classify: 2 concordant results from 3 tests

AOPs and Regulatory Use

Confidence vs. uncertainty | **AOP Continuum** | **USE**



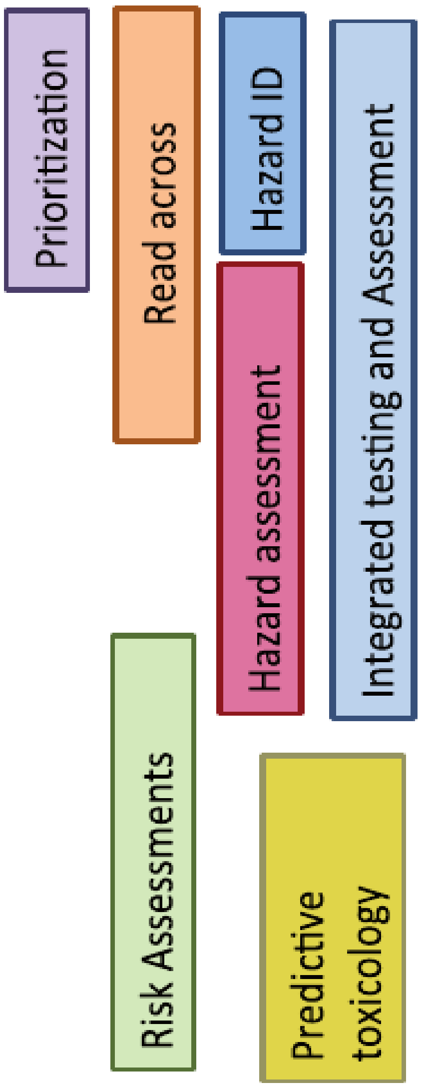
Correlative/qualitative
- mechanistic understanding of MIE/KE (Quantitative or not)
- simple statistical correlations with some biological plausibility between MIE/KEs and AOs

Qualitative
- some mechanistic understanding of linkages between MIE/KE and AO
- some evidence for causal linkages

Semi-quantitative
- some quantitative understanding
- dose-response information, toxicokinetics, metabolism

Quantitative
- Predictive causally-linked quantitative models
- Dose relationships
- Some understanding of intersecting pathways

Predictive system
- Quantitative understanding of relationships of intersecting pathways
- increased certainty of likelihood of a particular AO vs some other outcome



Possible AOPs for Carbon Nanotubes

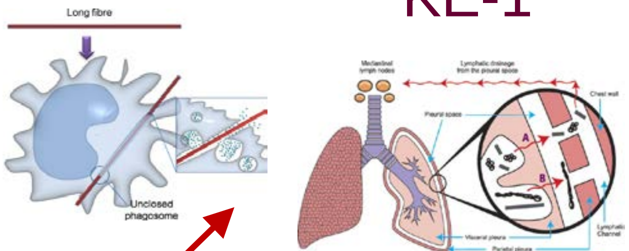
KE-1

KE-2, KE-3, etc

AOPs

CNT Properties

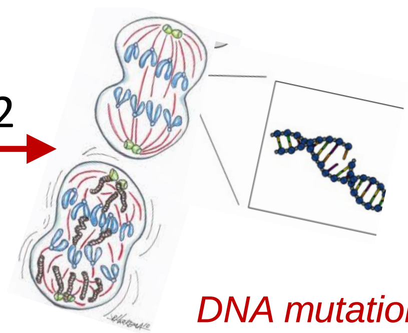
MIE-1



Pleural pore obstruction, frustrated phagocytosis

Mesothelioma

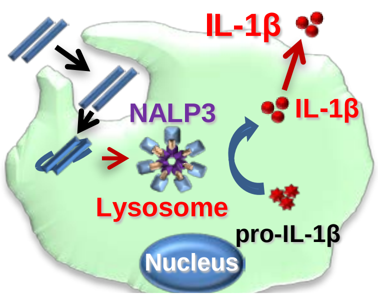
MIE-2



DNA mutation, mitotic spindle damage

Lung Adeno carcinoma

MIE-3



Lysosome damage, inflammasome

Chronic inflammation & Fibrosis

Integrated Approaches to Testing and Assessment
(inhalation exposures)

Is this CNT of concern to humans?

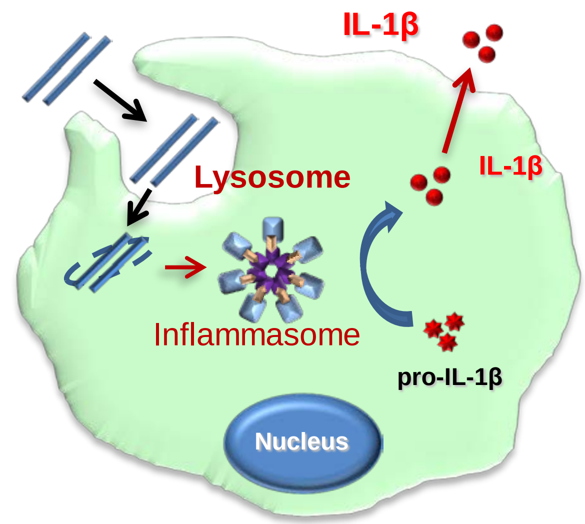
Yes, if it is inhalable

Is there potential for exposure?

Workplace
Consumer

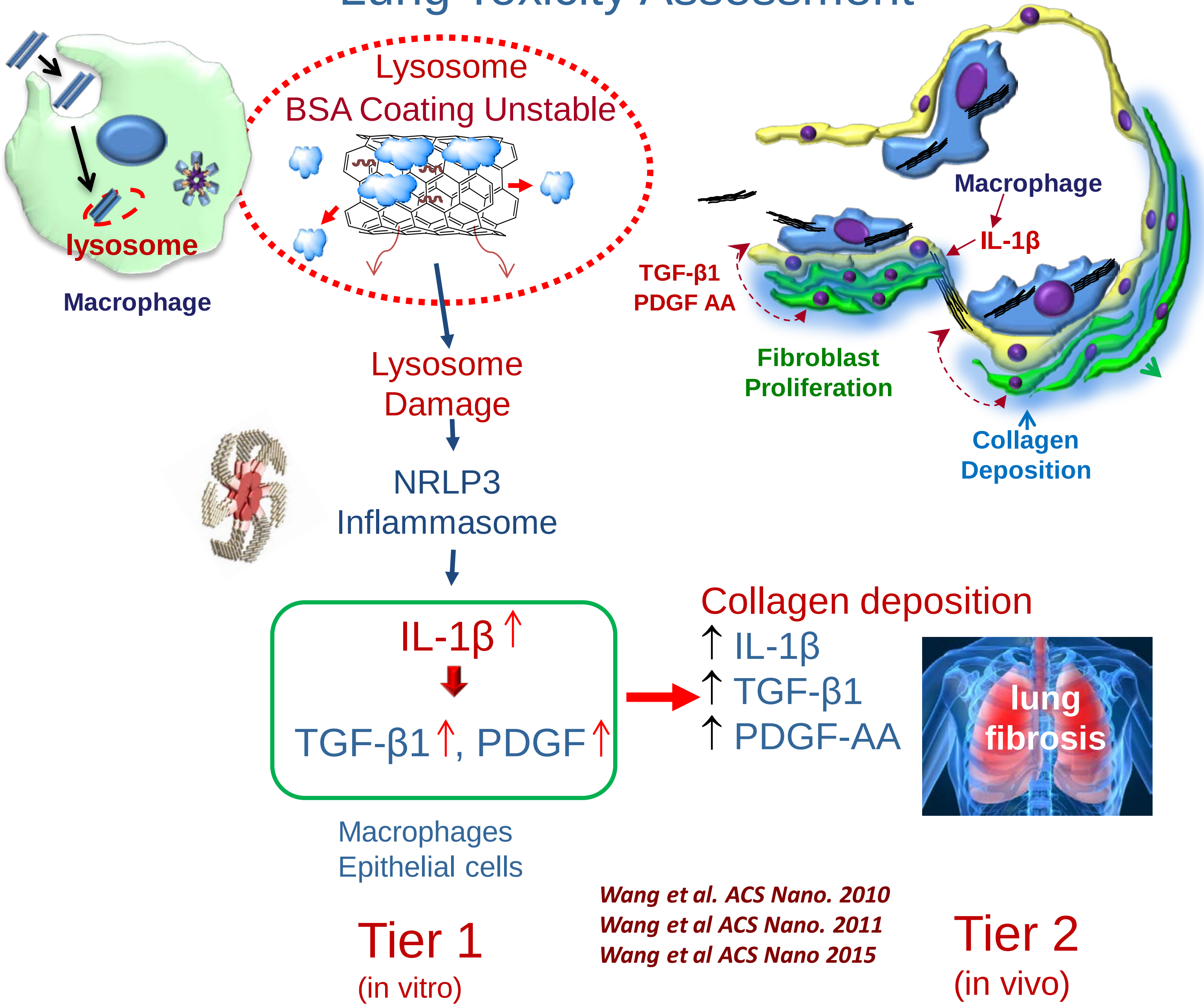
Is the CNT toxic?

“data”



MIE
AOPs –mechanistic
Tiered Testing
IATA

Predictive Toxicological Paradigm for CNT Lung Toxicity Assessment

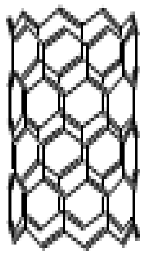


Wang et al. ACS Nano. 2010
Wang et al ACS Nano. 2011
Wang et al ACS Nano 2015

Comparison of MWCNT Surface Charge to validate that Cellular Assays (Tier 1) reflect Lung Injury Outcome (Tier 2)

(not logistically feasible to test this in a 90 day study)

#1 Crude

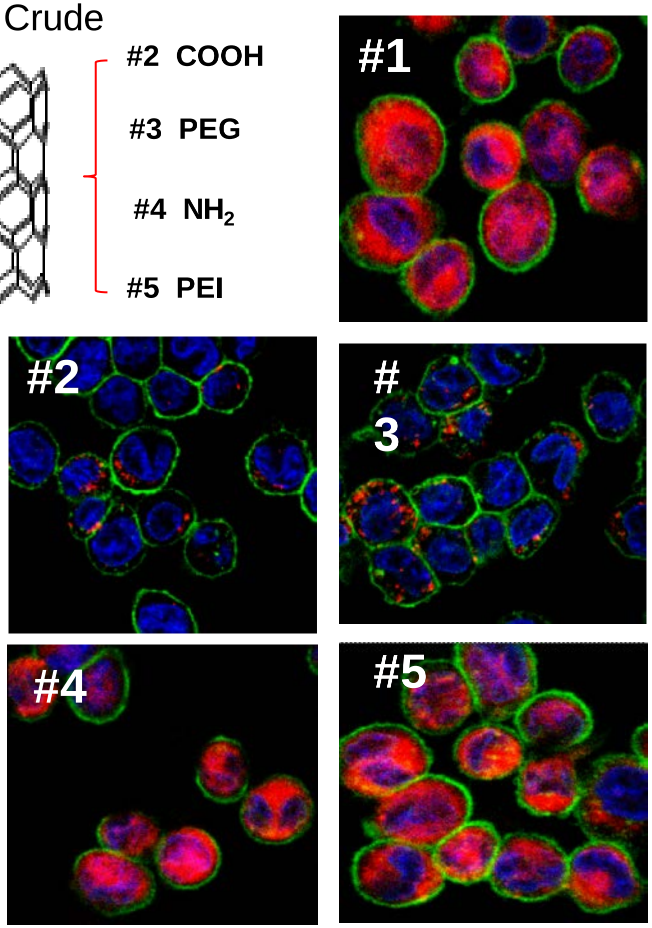


#2 COOH

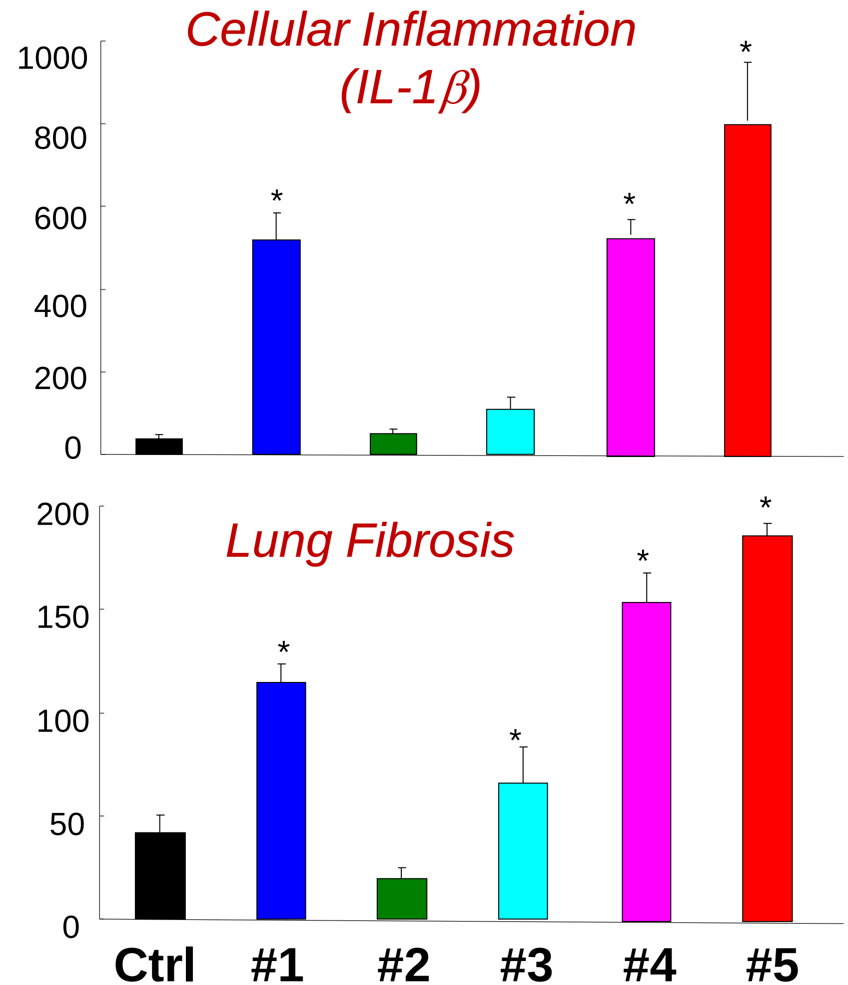
#3 PEG

#4 NH_2

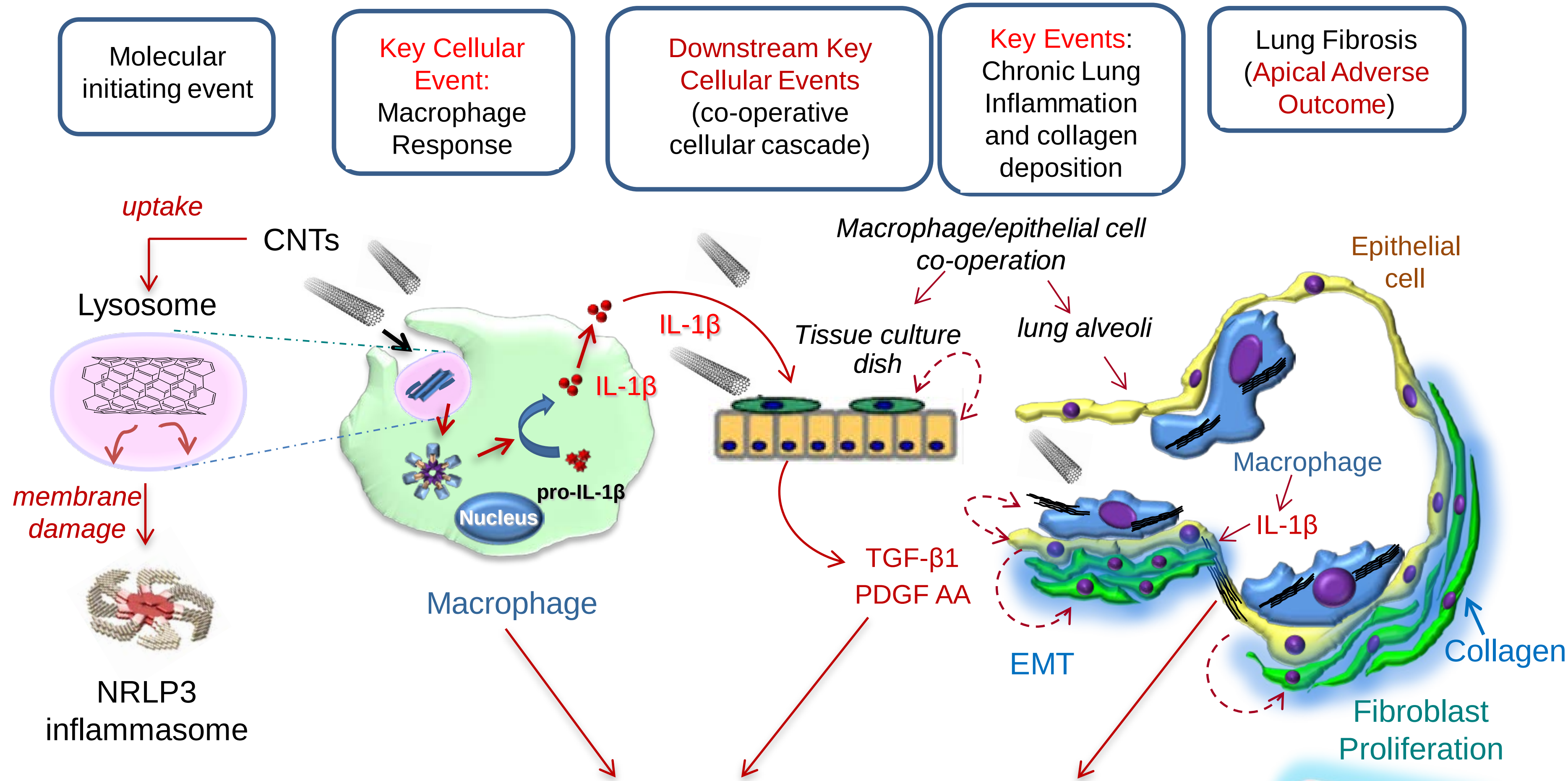
#5 PEI



Confocal to show Lysosomal damage



Li R. et al ACS Nano. 2013



CNT Tiered Testing Approach

Tier 1 Testing (ATS)
(macrophages, macrophage/epithelial co-culture)

IL-1β
TGF-β1
PDGF

Tier 2 Testing
(short term animal exposure)

AOP-based
Histology (fibrosis)
Lung collagen content
Lavage fluid cytokines

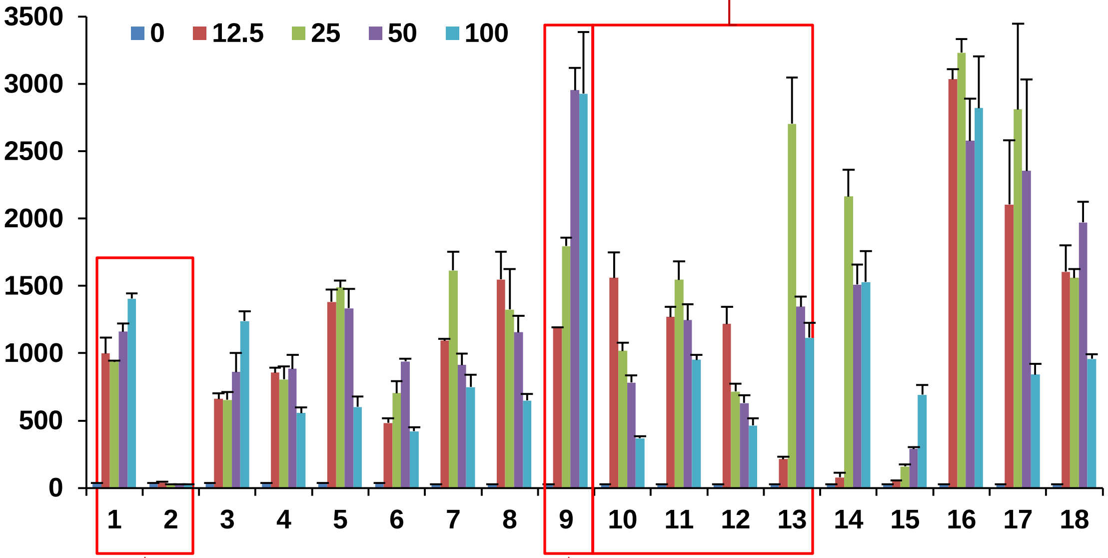
90-Day Inhalation
(Tier 3)

Descriptive apical outcome, non-mechanistic

Nel & Malloy. Science. 2017

Tier 1 Testing of 18 CNTs: US, China and EU

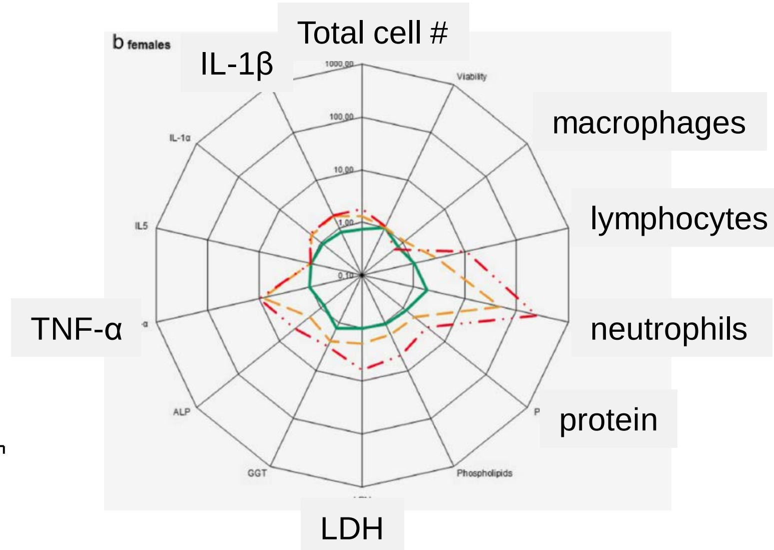
Tier 1: IL-1β values (pg/ml)



CEIN Ref materials

Mitsui

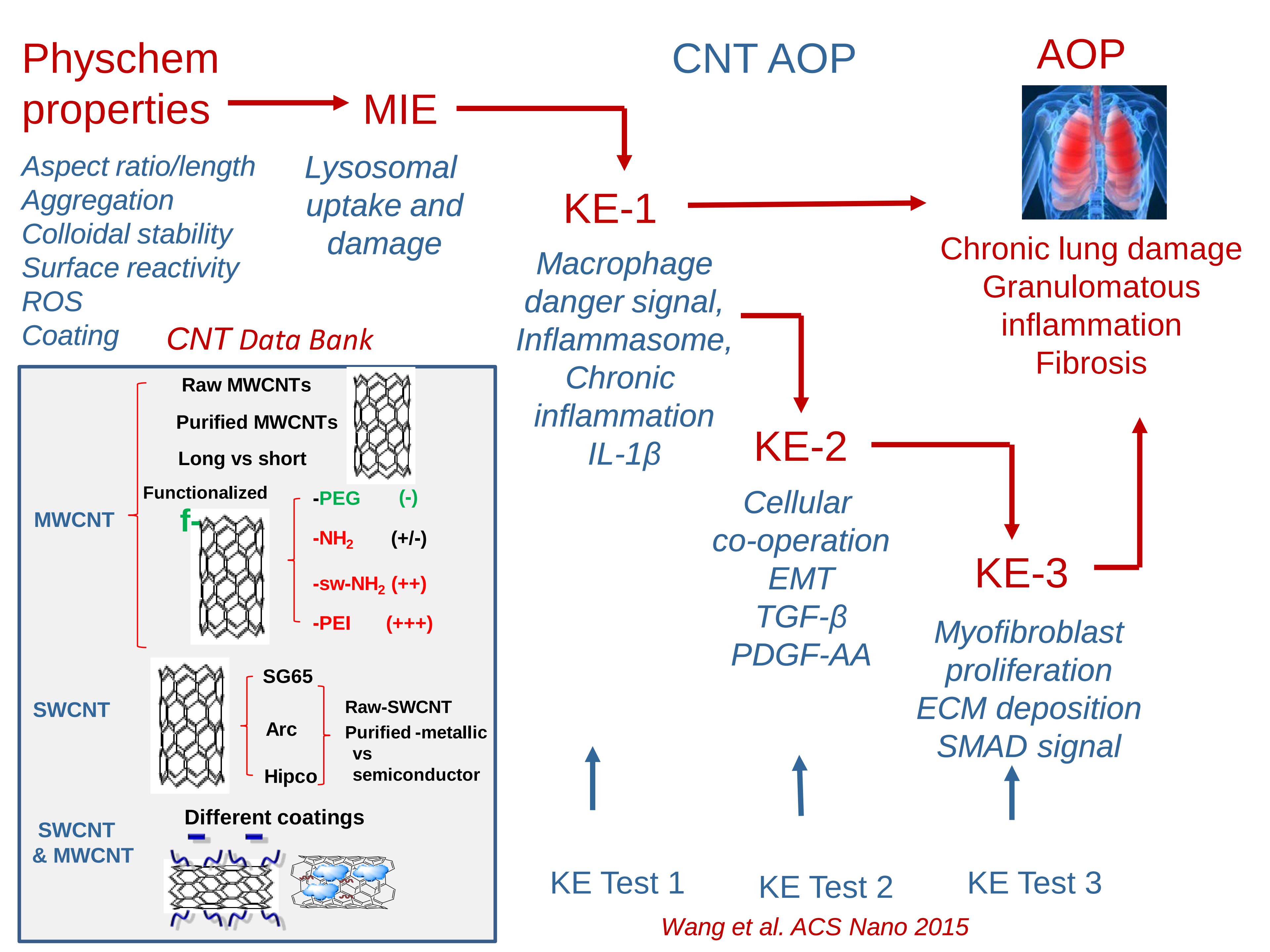
Tier 3: 90 day inhalation study (EU Materials)



2015 Pothmann

1	As-purchased (AP) crude MWCNT
2	Pluronic F108 coated AP-MWCNT (neg control)
3	MWCNT-COOH (XXX)
4	XXX (MWCNT)
5	XXX (MWCNT)
6	XXX (SWCNT)
7	XXX (SWCNT)
8	XXX (MWCNT)
9	Mitsui (MWCNT-7)

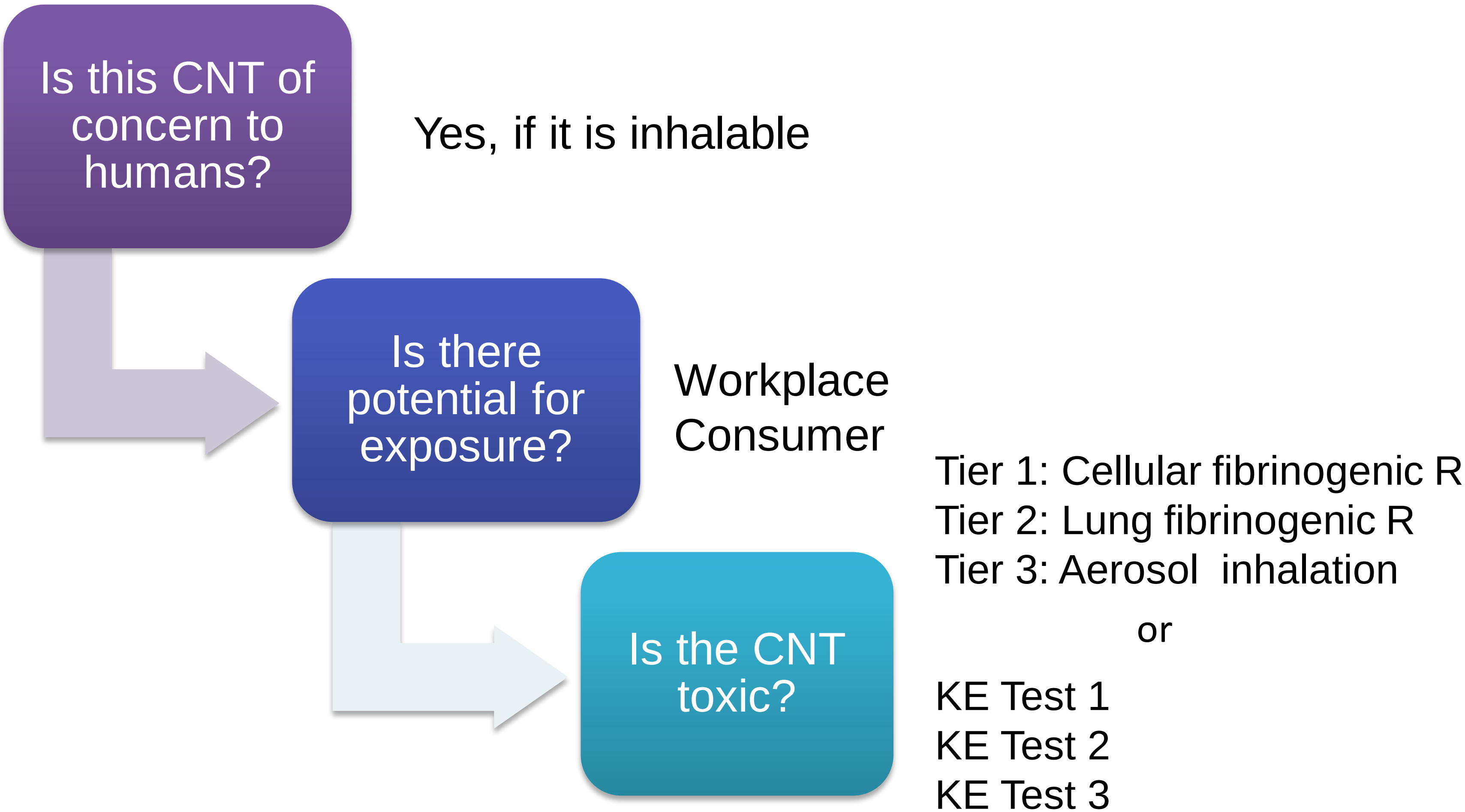
10	EU NM- XXX (MWCNT)	NanoReg JRC
11	EU NM- XXX (MWCNT)	
12	EU NM- XXX (MWCNT)	
13	EU NM- XXX (MWCNT)	
14	XXX MWCNT (China)	
15	Short carboxyl MWCNT (China)	
16	Long carboxyl MWCNT (China)	
17	SWCNT (China)	
18	SWCNT (China)	



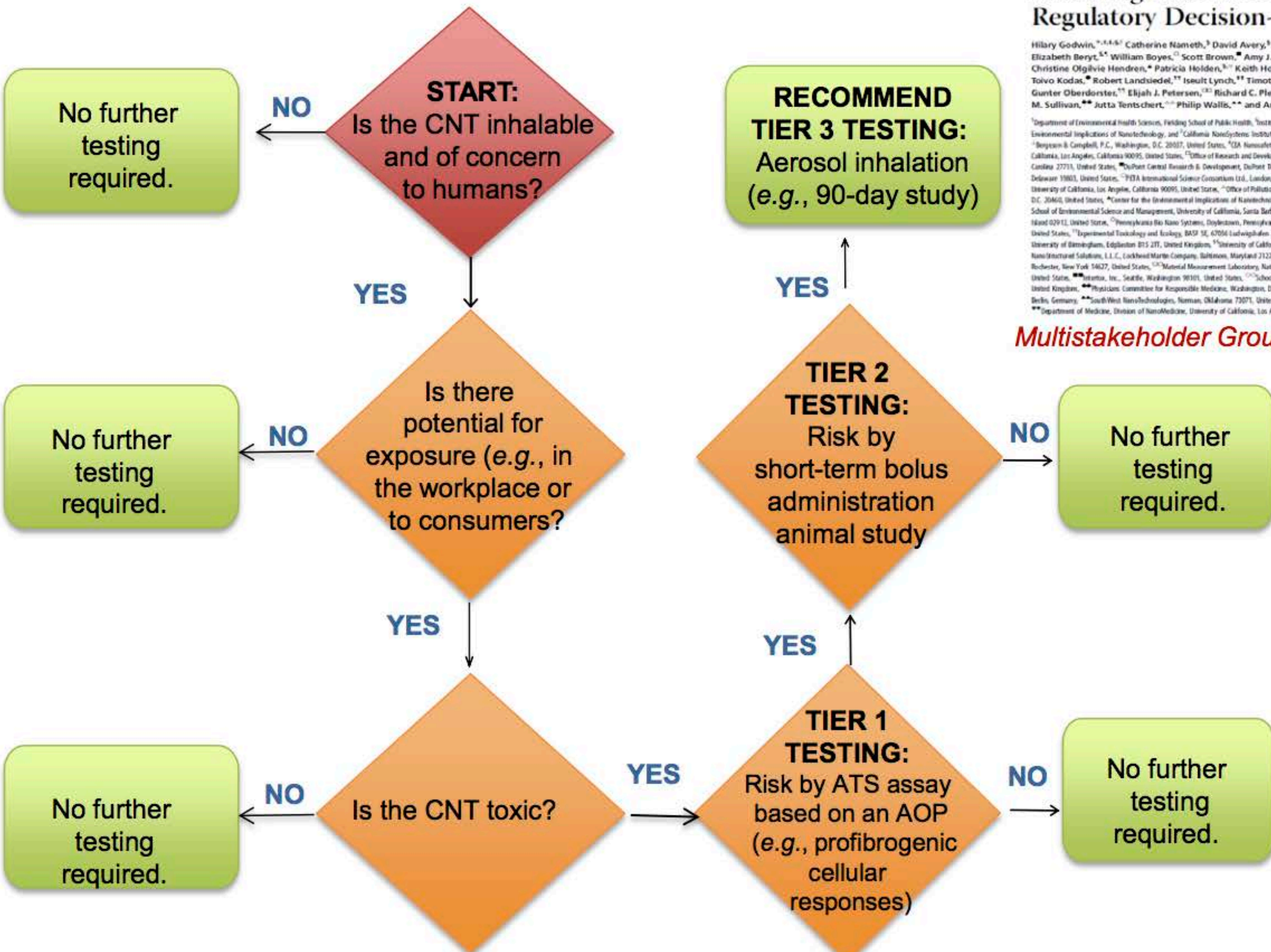
Physchem properties
MIE
CNT AOP
AOP
Aspect ratio/length
Aggregation
Colloidal stability
Surface reactivity
ROS
Coating
Lysosomal uptake and damage
KE-1
Macrophage danger signal, Inflammasome, Chronic inflammation IL-1β
Chronic lung damage
Granulomatous inflammation
Fibrosis
CNT Data Bank
MWCNT
Raw MWCNTs
Purified MWCNTs
Long vs short
Functionalized
f-
-PEG (-)
-NH2 (+/-)
-sw-NH2 (++)
-PEI (+++)
SWCNT
SG65
Arc
Hipco
Raw-SWCNT
Purified -metallic vs semiconductor
SWCNT & MWCNT
Different coatings
KE-2
Cellular co-operation
EMT
TGF-β
PDGF-AA
KE-3
Myofibroblast proliferation
ECM deposition
SMAD signal
KE Test 1
KE Test 2
KE Test 3
Wang et al. ACS Nano 2015

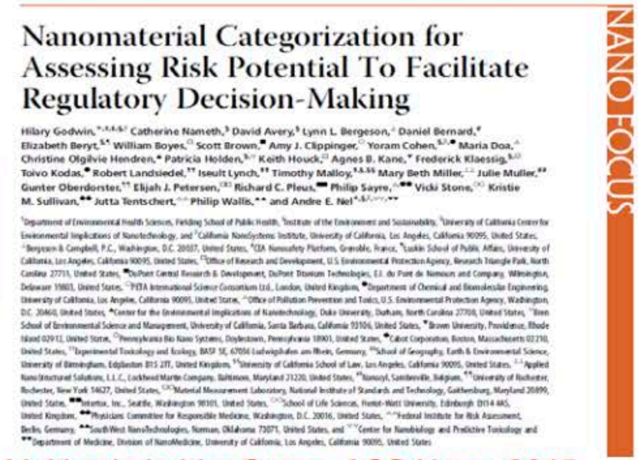
Integrated Approaches to Testing and Assessment (IATA) framework for CNTs

(Inhalation exposure)



Proposal how to incorporate ATS data in a decision-tree to categorize CNTs by a tiered testing approach





NANO FOCUS

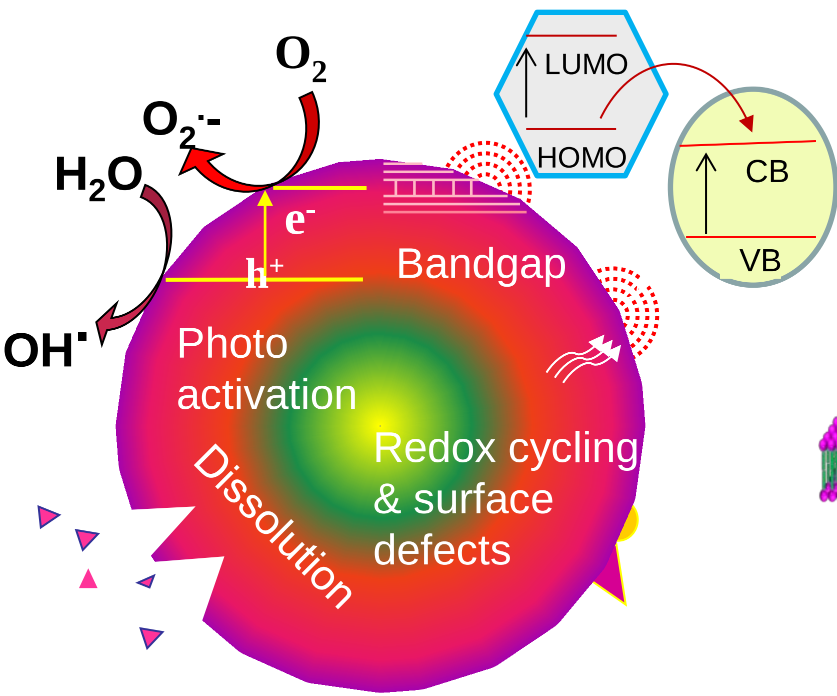
Nanomaterial Categorization for Assessing Risk Potential To Facilitate Regulatory Decision-Making

Hilary Godwin, Catherine Nameth, David Avery, Lynn L. Bergeson, Daniel Bernard, Elizabeth Beryt, William Boyes, Scott Brown, Amy J. Clippinger, Yoram Cohen, Maria Doa, Christine Olgilvie Hendren, Patricia Holden, Keith Houck, Agnes B. Kane, Frederick Klaessig, Toivo Kodas, Robert Landsiedel, Iseult Lynch, Timothy Malloy, Mary Beth Miller, Julie Muller, Gunter Oberdorster, Elijah J. Petersen, Richard C. Pleus, Philip Sayre, Vicki Stone, Kristie M. Sullivan, Jutta Tentschert, Philip Wallis, and Andre E. Nel

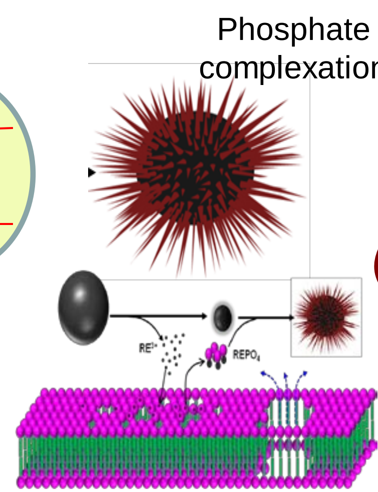
Multistakeholder Group. ACS Nano. 2015

Metal Oxide MIE's and AOPs in Lung Toxicity

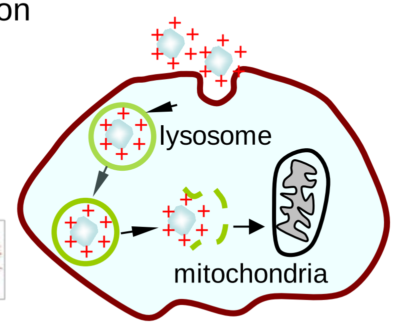
Soluble Metal oxides	Transition MOX's		Rare Earth Oxides	High Aspect Ratio	Surface reactivity (silanols)
ZnO CuO	Fe_2O_3 Fe_3O_4 WO_3 TiO_2 Al_2O_3 SiO_2	CoO Co_3O_4 Cr_2O_3 Mn_2O_3 Ni_2O_3	**CeO_2, Eu_2O_3 Gd_2O_3 HfO_2, La_2O_3, Sb_2O_3, Sb_2O_3, SnO_2, Yb_2O_3, Er_2O_3 Y_2O_3 ZrO_2**	Spherical vs nanorod CeO_2 & TiO_2	SiO_2 -fumed -amorphous -mesoporous -crystalline



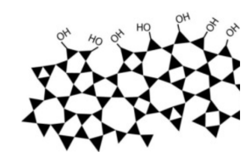
Disrupt Redox equilibrium
Oxidative stress



NRLP3 inflammasome
Autophagy interference



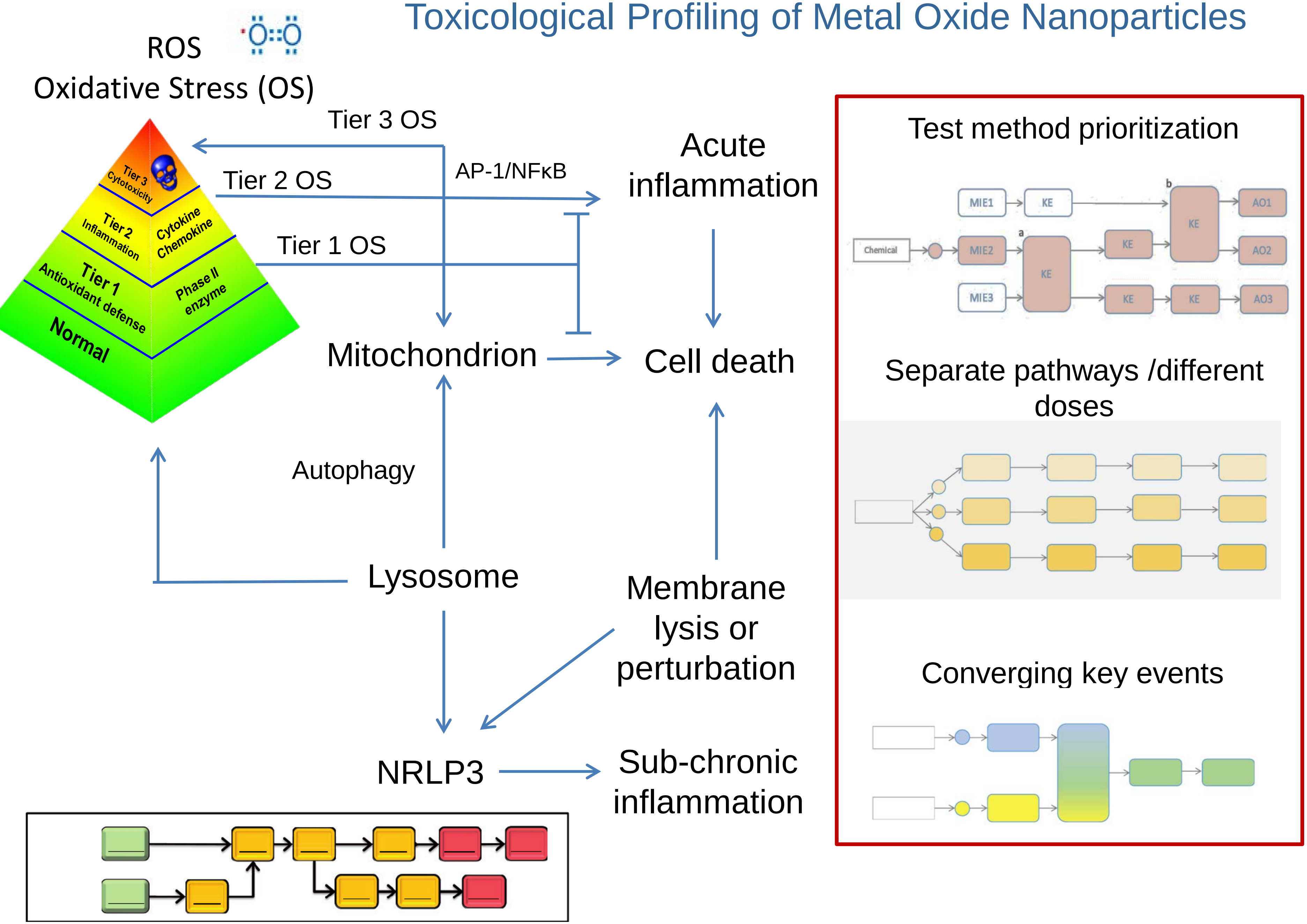
NRLP3 inflammasome
Sub-acute inflammation



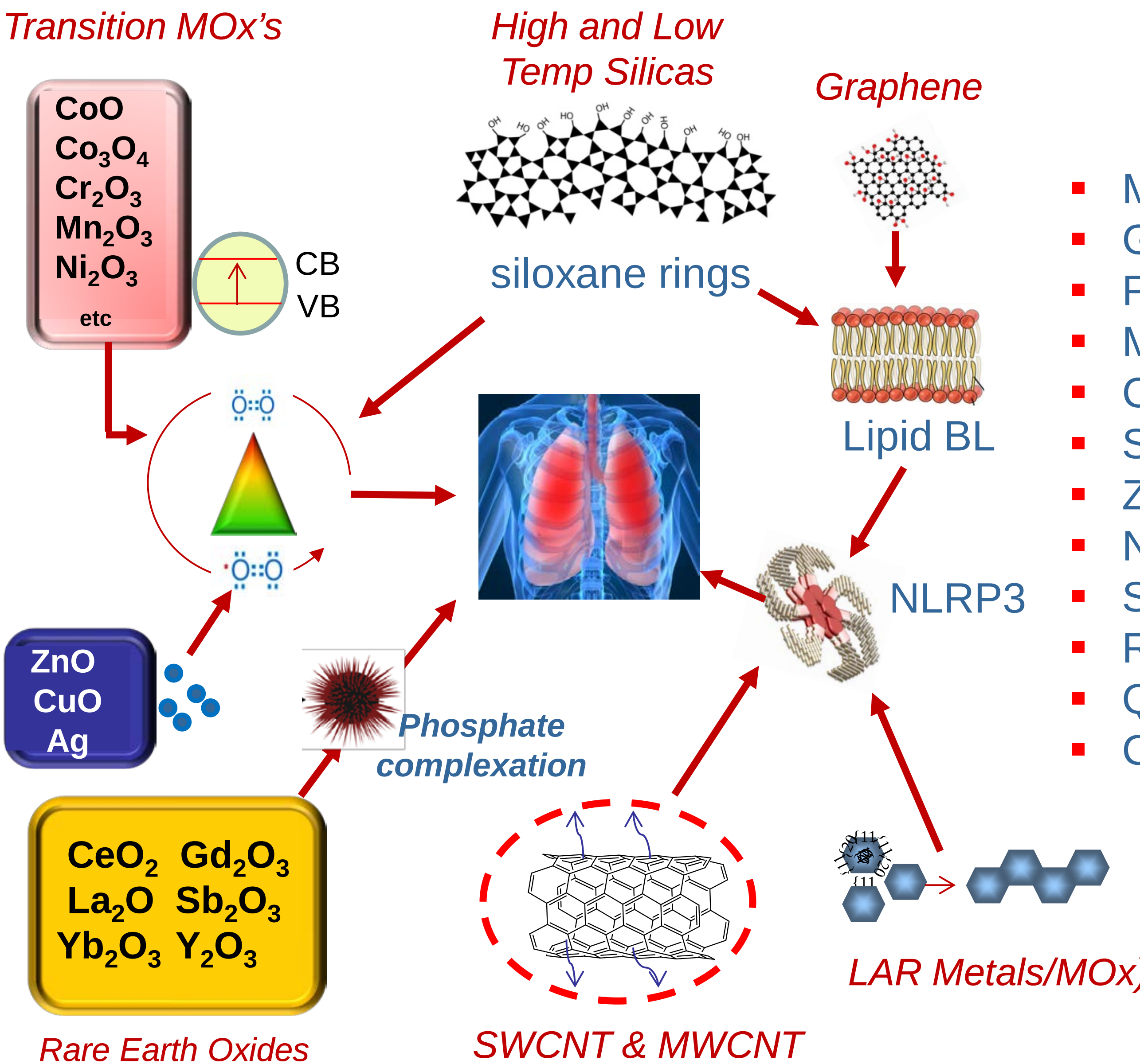
Membrane lysis (acute)
NRLP3 (sub-acute)

Nel et al. Science 2006
Nel et al. Nature Materials. 2009

Use of Individual or Converging AOPs for the Toxicological Profiling of Metal Oxide Nanoparticles



Predictive Toxicology Approaches allows Large Numbers of Materials to be Profiled for Inhalation Tox Decisions

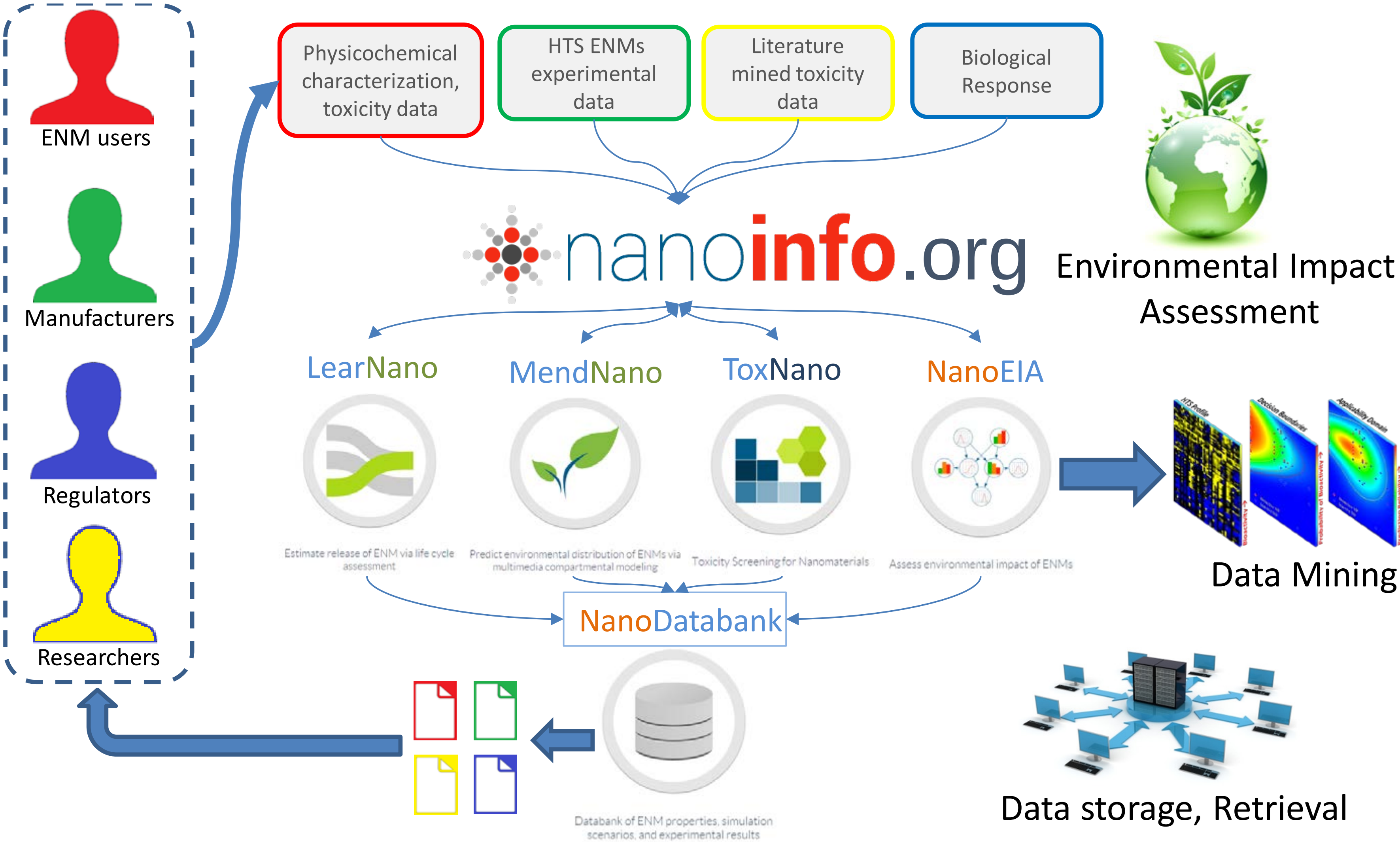


- MWCNTs, SWCNTs
- Graphene
- Fullerenes
- Metal Oxides
- Commercial Cu and CuO
- Silica and Fumed silica
- ZnO
- Nano Ag
- Semiconductor III-V, Mox's
- REOs
- Quantum dots
- Composites/Release

George e al. ACS Nano. 2010
Xia et al. ACS Nano. 2011
Zhang et al. ACS Nano. 2012
Nel et al. ACR. 2012

NanoDatabank, Data Analytics and NanoEHS Decision Support Tools (www.nanoinfo.org)

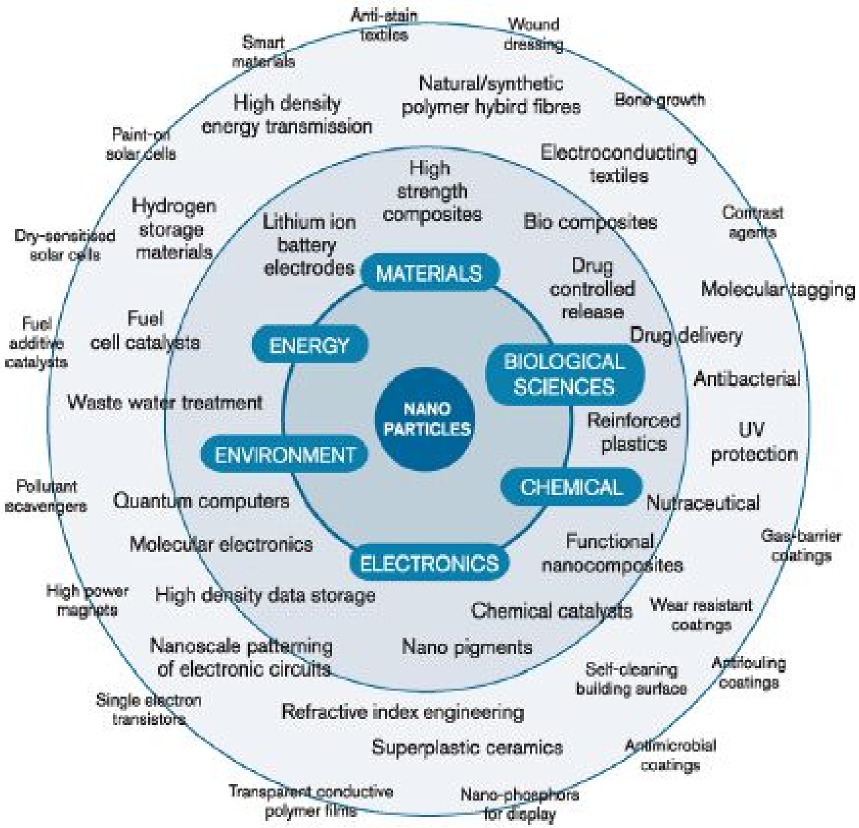
Input raw Data, shared findings using Centralized Nanodatabank
Estimate releases of ENMS, fate & transport analysis, analyze HTS data, toxicity predictions and decision analysis support tools

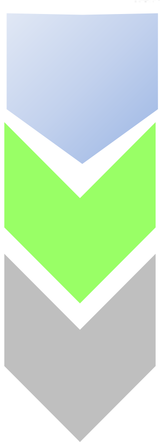


Pitfalls of Alternative Test Strategies

- *ATS* do not comprehensively capture biological processes in the context of intact organs or whole organisms
- *In vitro/in vivo* disconnect (*offset by AOPs as intellectual constructs)*
- Biased or incomplete coverage of injury response pathways
- False positives and false negatives
- Does not cover toxicokinetics and ADME
- No real-life exposure scenarios, including use of unrealistic dosimetry
- Only tests acute toxicological events, not chronic or repetitive exposures

Rapid Evolution in Current Nano EHS Regulatory Assessment needs: Pristine vs Nano-enabled Products





Nanomaterials

Nano-intermediates

Nano-enabled Products

New EPA Section 8 Reporting Rule triggering reporting PhysChem Data (Jan 2017)

- Size
- Coating
- Shape
- Zeta potential
- Surface area
- Dispersion stability
- Surface reactivity

Expanded range of Industry Actors due to the new rule

- Grinding
- Mixing
- Formulating
- Dispersing
- Surface coatings
- Dispersion stability
- Surface reactive materials

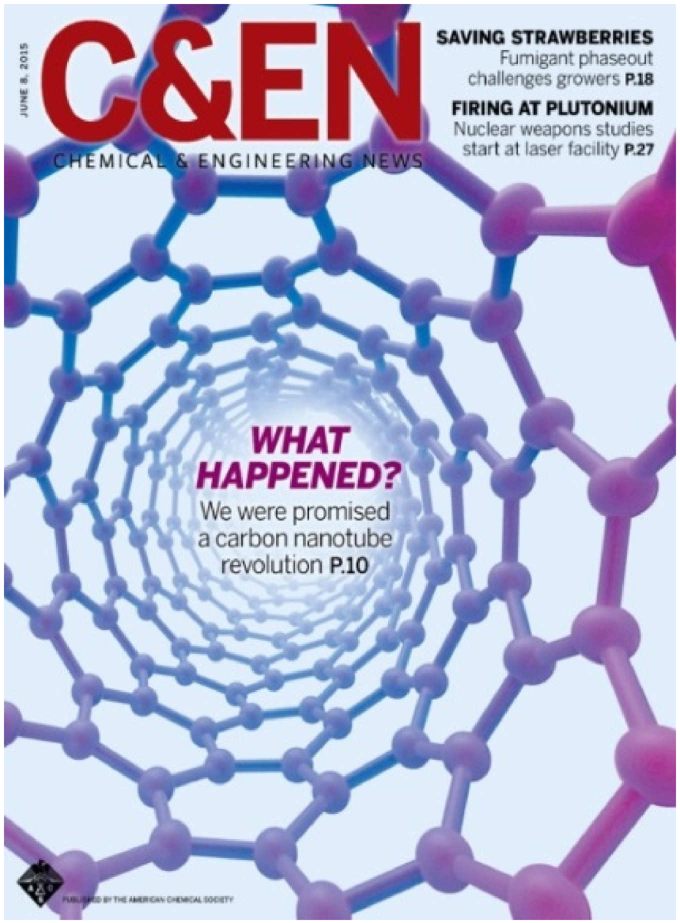
CEIN Broad Capabilities for Implementing the use of ATS for Nano EHS Assessment and Governance

- Predictive toxicological profiling of individual and broad material categories (data bank, comparison grid, modeling)
- Use of AOPs and HTS to assist data collection for IATA and regulatory decision making
- Assist industry in developing new integrated test strategies for emerging nano-enabled materials and nano-composites
- Tailor experimentation to relevance of potential exposures
- Safer design principles based on structure-activity analysis
- Simulations, modeling, LCA

ATS at the R&D and Design Stage

- Demonstrate whether change in manufacturing process alters biological response
- Adjust surface chemistry or coating on “new grade” material to match registered “base” material
- Demonstrate “equivalency” to an approved competitor product (or benchmark materials)
- Could examine “extremes” in a CNT-family to develop ranges on which SNURs are based
  - E.g., Company X 12 MWCNTs (4 PMN-numbers)

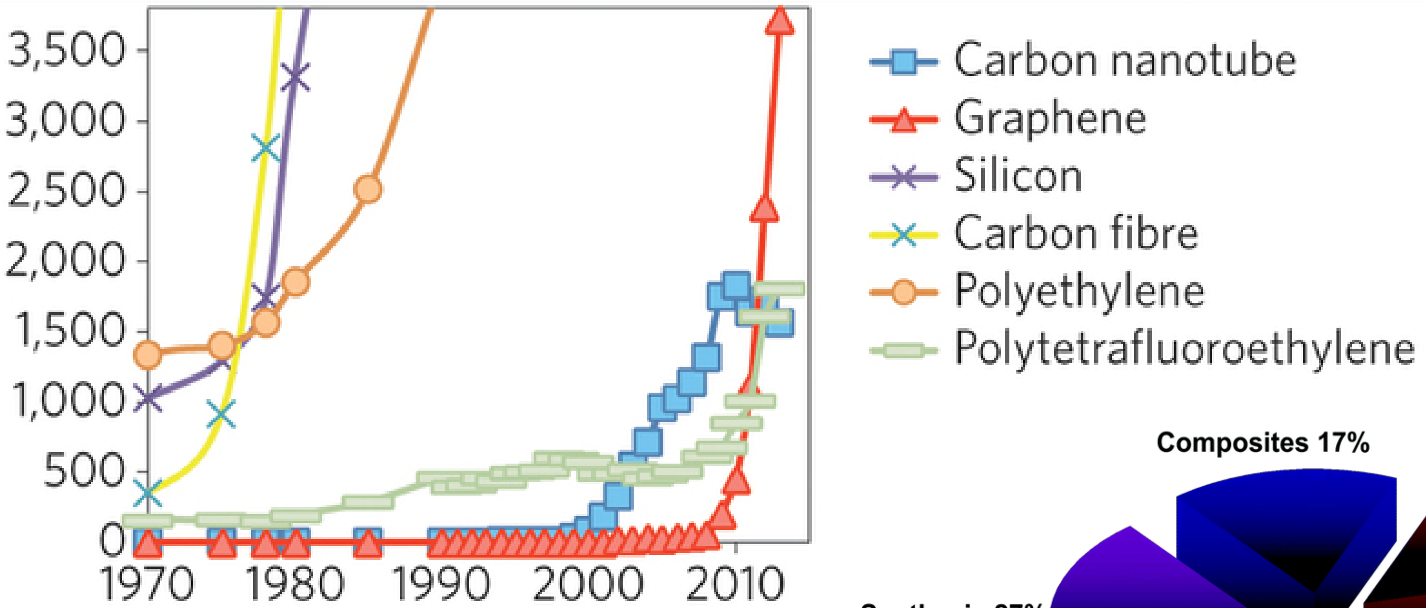
The Story about the CNT Commercial Enterprise



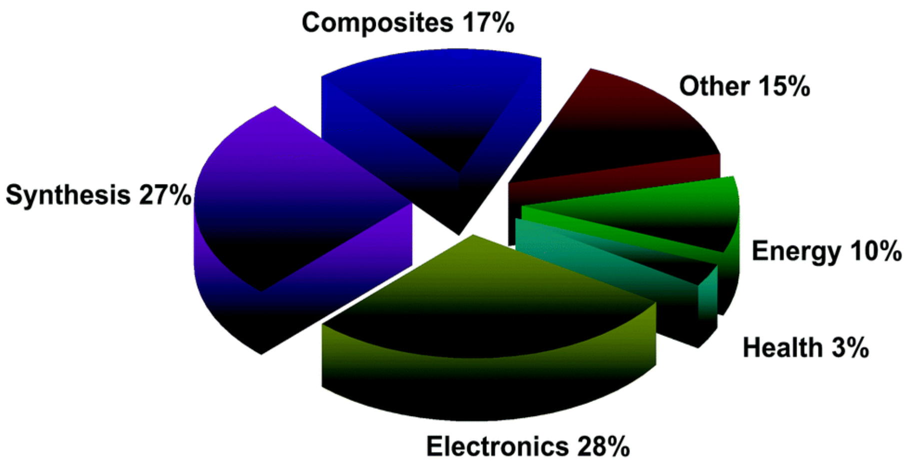
(2015)

- Over expectation/hype
- Over production relevant to demand
- Technical issues: purity, crosslinking
- Incorporation into other products
- Risk avoidance
- Risk perception: “the next asbestos”

Will History Repeat Itself for Graphene?



Nature Nanotechnology, October, 2014

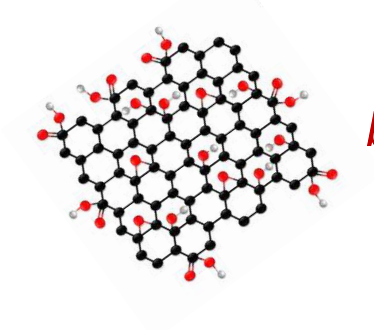


The trajectory of graphene is following a similar path to carbon nanotubes with patents being filed at an even quicker rate.

Graphene SAR Analysis as the basis for ATS, AOPs and Predictive Toxicological Paradigms

Planar surface hydrophobicity

Hydrophilic edge effects, Flake sizes



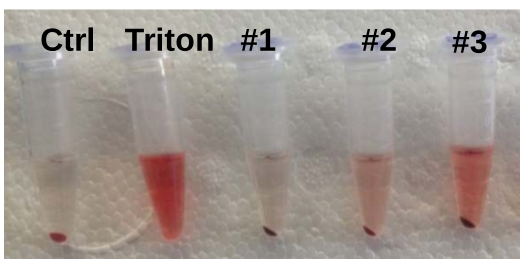
Graphene flakes behave as colloids as well as 2D planar surfaces

Planar surface hydrophobicity

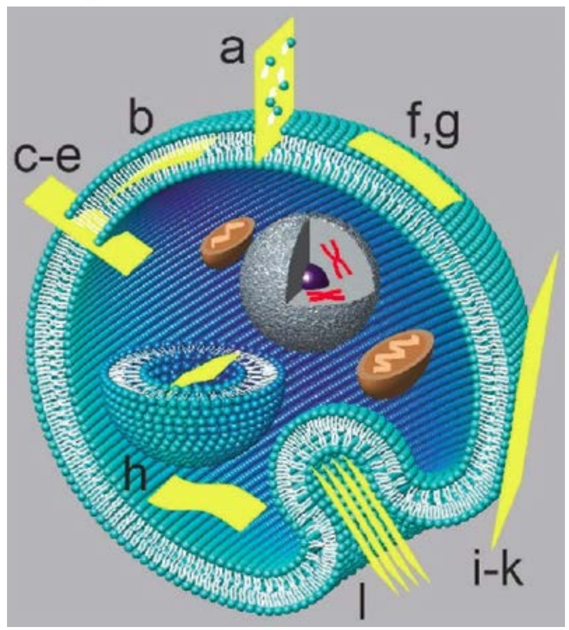
Membrane damaging AOPs (for ATS)

Surface functionalities (COOH, Epoxy, OH)

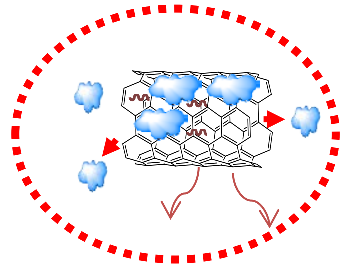
Carbonyl radicals



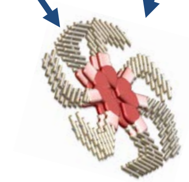
Membrane lysis
Lipid peroxidation
Membrane destructive lipid extraction



Cellular uptake



*Lysosomal **AOP***



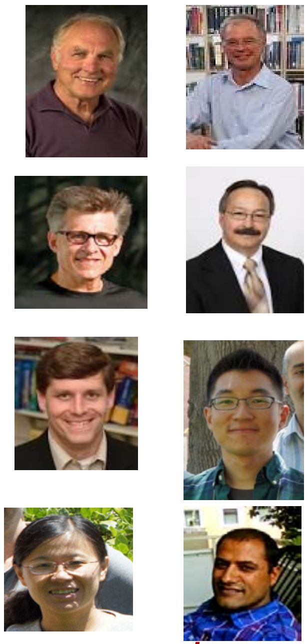
Inflammasome

IL-1β
(CNT Tier 1)

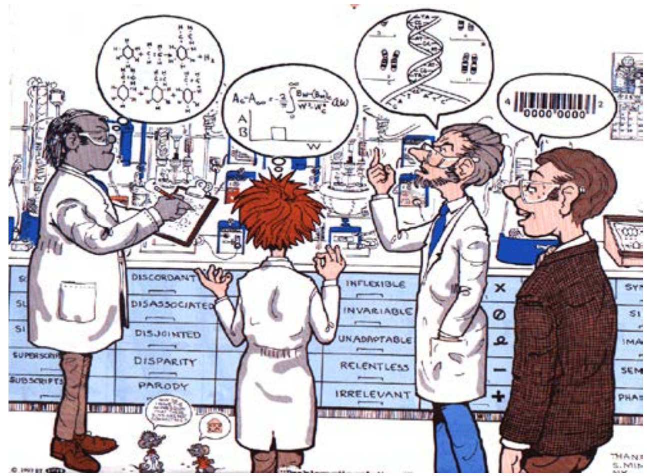
Wang et al ACS Nano 2015
Hurt R et al Chem Soc Review 2016
Hersam Lab North-Western

UC CEIN - A Multidisciplinary Workforce

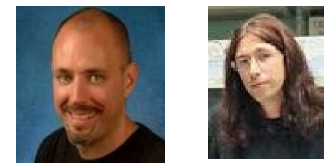
Chemistry/
Material Science



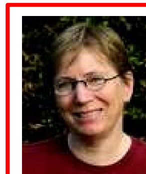
et. al



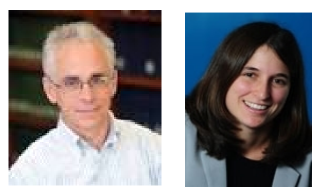
MSSR/
High Throughput



Social
Science

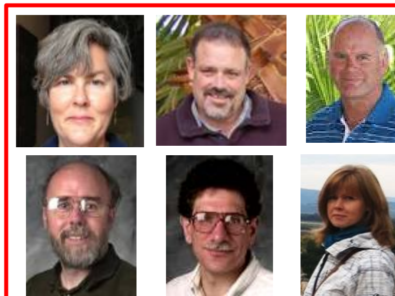


Law/Policy



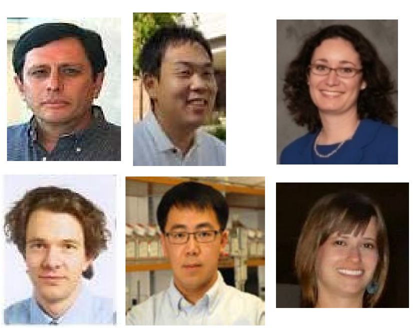
Public Health

Ecology/
Environmental Science



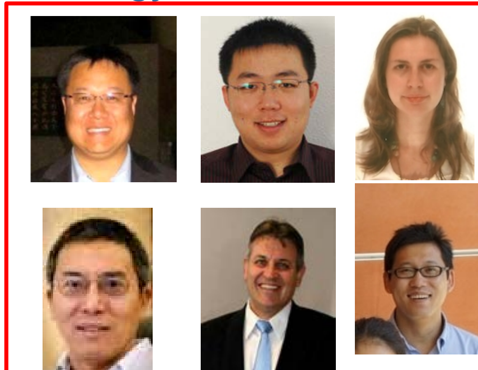
et. al

Engineering



et. al

Biology/Tox/Medicine



et. al

Computational Modeling

et. al