The California NanoSystems Institute (CNSI)

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

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

Nano EHS

Hazard Identification

Exposure Assessment

Dose-Response Assessment

Risk Characterization

Little quantitative RA
Some qualitative RA
OELS
Tiered risk assessment
Integrated approaches to decision making (IATA)
Chemical Toxicity Principles not Optimal for Molecular Initiation or Triggering Events but useful for AOP assessment

Carbon as an example.....

Butane $C_4H_{10}$

\[
\begin{align*}
&H & & & & & H \\
&H & & & & H & & H \\
H & C & C & C & C & H \\
H & H & H & H & & & & 
\end{align*}
\]

Isobutane $C_4H_{10}$

\[
\begin{align*}
&H & & & & & H \\
&H & & & H & & H \\
&H & C & C & C & C & H \\
&H & H & & H & & & & 
\end{align*}
\]

Graphene 2004

Many undiscovered allotropes for example $sp-sp^{2}$-graphyne 20??

Carbon nanotubes 1991

Fullerenes 1985

Risk Contexts

Chemical Characterization

Toxicity Testing

Targeted Testing

Population and Exposure Data

Dose-Response and Extrapolation Modeling
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 $\zeta$-potential
    - Corona
    - Dissolution
    - Surface reconstruction
    - Sorption

Emergent properties
- 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)

Tox- SARs approximately associated with injury

*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

Adverse Outcomes in Intact Animals (limited screening capacity)

Confirm In vivo Hazard potential

ENM Libraries of different composition and accentuated Physchem Properties

Cellular or Bio-molecular Endpoints (High content screening)

SARs (pathophysiology of disease)

SARs (molecular events nano/bio interface)

Dosimetry

Pathways Of Toxicity

Dosimetry

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
Risk Identification and decision making to:
- Reduce risk
- Influence governance
- Dosimetry calculations
- Safer design

In vivo hazard ranking

High toxicity
Moderate
Low toxicity (nuisance dust)

Pulmonary inflammation

Dose (mass, surface area dose, reactive surface area)

Organism
Animal testing

Prioritize
Compare
Speed up
Validate
Dosimetry
Refine

Similar behavior (Cluster)

Cells, bacteria, yeasts, zebrafish embryos

In silico decisions, in vitro ranking

High throughput screening

Nanomaterial libraries

Compositional
Metal Oxides
Metals
CNTs

Property accentuation
Size, Shape, AR
Dissolution
Band gap

Commercial nanoproducts

Organization for Predictive Toxicological Research UC CEIN

6/2/2015
Tools to establish Predictive Models

High Throughput Screening

Composition and Combinatorial ENM Libraries

Surface Charge

Size

Shape

AR

Surface Functionalization

Surface chemistry

Dissolution chemistry

Crystal Structure

Band Gap

Epifluorescence

Heat map ranking

Pathways of Toxicity

Oxidative stress

Epifluorescence

Computational analysis

Composition and Combinatorial ENM Libraries

1h 24h

PI Ca O$_2^-$ H$_2$O$_2$ MMP

ZnO

Oxidative stress
From Pathways of Toxicity to Adverse Outcome Pathways

Redox activity and ROS e.g., TiO₂, CuO, CoO

Dissolution, shedding toxic ions, e.g., ZnO, CuO

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

Membrane Lysis e.g., fumed SiO₂,

Inflammasome activation e.g., High aspect ratios

Metal → Metal ions

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

Inflammasome

Nucleus

NALP3

pro-IL-1β

IL-1β

O₂ → O₂

O₂

h⁺
e⁻

SH

SS

Conduction Band

Valence Band

hv

ΔE₀

Metal

Metal ions

lysosome

mitochondria

Nucleus

Cell membrane

From Pathways of Toxicity to Adverse Outcome Pathways

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
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
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)

Example Cosmetic Chemical Test Series:
- Non-cosmetic chemicals
- Actives
- Dyes
- Fragrances
- Preservatives
- Surfactants

Key Events

**KE1**: Direct Peptide Reactivity Assay
No

**KE2**: KeratinoSens™ Oxidative Stress Assay
Yes

**KE3**: Antigen Presenting Cell Activation Assay

Classify: 2 concordant results from 2 tests

Classify: 2 concordant results from 3 tests
AOPs and Regulatory Use

AOP Continuum

**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

USE

- Prioritization
- Read across
- Hazard ID
- Integrated testing and Assessment

Confidence vs. uncertainty

Model Uncertainty

Confidence in using the AOP for quantitative prediction of AO
Possible AOPs for Carbon Nanotubes

KE-1

KE-2, KE-3, etc

AOPs

Mesothelioma

Lung Adeno carcinoma

Chronic inflammation & Fibrosis

Properties

CNT

MIE-1

Pleural pore obstruction, frustrated phagocytosis

MIE-2

DNA mutation, mitotic spindle damage

MIE-3

Lysosome damage, inflammasome

KE-1

KE-2, KE-3, etc

MIE-1

MIE-2

MIE-3
Integrated Approaches to Testing and Assessment (inhalation exposures)

Is this CNT of concern to humans?
Is there potential for exposure?
Is the CNT toxic?

Yes, if it is inhalable

Workplace Consumer

MIE AOPs –mechanistic Tiered Testing IATA

IL-1β
Inflammasome
pro-IL-1β
Lysosome
Nucleus

“data”
Predictive Toxicological Paradigm for CNT Lung Toxicity Assessment

Lysosome Damage
- BSA Coating Unstable
- IL-1β↑
- TGF-β1↑, PDGF↑

NRLP3 Inflammasome
- IL-1β↑
- TGF-β1↑, PDGF↑

Collagen deposition
- IL-1β↑
- TGF-β1↑
- PDGF-AA↑

Tier 1 (in vitro)
- Macrophages
- Epithelial cells

Tier 2 (in vivo)
- Wang et al. ACS Nano. 2010
- Wang et al ACS Nano. 2011
- Wang et al ACS Nano 2015

Macrophages
- IL-1β
- TGF-β1
- PDGF-AA

Fibroblast Proliferation
- Collagen Deposition

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 NH₂
- #5 PEI

Confocal to show Lysosomal damage

**Cellular Inflammation** (IL-1β)

**Lung Fibrosis**

- Li R. et al ACS Nano. 2013
CNT Tiered Testing Approach

Nel & Malloy. Science. 2017
Tier 1 Testing of 18 CNTs: US, China and EU

Tier 1: IL-1β values (pg/ml)

Tier 3: 90 day inhalation study (EU Materials)

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)
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)

NanoReg JRC

2015 Pothmann
Integrated Approaches to Testing and Assessment (IATA) framework for CNTs

(Inhalation exposure)

Is this CNT of concern to humans?

Yes, if it is inhalable

Is there potential for exposure?

Workplace
Consumer

Tier 1: Cellular fibrinogenic R
Tier 2: Lung fibrinogenic R
Tier 3: Aerosol inhalation

or

KE Test 1
KE Test 2
KE Test 3

Is the CNT toxic?
Proposal how to incorporate ATS data in a decision-tree to categorize CNTs by a tiered testing approach

START: Is the CNT inhalable and of concern to humans?

RECOMMEND TIER 3 TESTING: Aerosol inhalation (e.g., 90-day study)

Tier 2 Testing: Risk by short-term bolus administration animal study

Tier 1 Testing: Risk by ATS assay based on an AOP (e.g., profibrogenic cellular responses)

No further testing required.

Is the CNT toxic?

No further testing required.

Is there potential for exposure (e.g., in the workplace or to consumers)?

No further testing required.

Multistakeholder Group. ACS Nano. 2015

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

Hillary Goddard,1,4,* Christa Nemes,1,4,† Daniel Avra,1 Lynn L. Bergstrom,1 Daniel Bernard,1 Elizabeth Avery-Akeneri,1 Karin A. Virkkunen,1 Scott Brown,1 John J. Sturgeon,1 Aurelia O. Oluwafeline,1 Carlielle Corbin,1 Becca Wright,1 Christine O'Grady,1 Ashley H. Reed,1 Sarah Church,1 Valeria Lanforte,2,3,4* Ethan Hirsch,1* Stephen Reay,1* Richard R. Schrock,1* Udo Schulte-Frohlinde,5* Jennifer L. Kipen,1* James L. Thomas,1* Eduardo A. Miranda,1* Hyun-Jae Lim,1* Adam C. McCorkle,1* Rachel E. Bollinger,1* Paul L. Tomsia,1* Juan R. Undritz,1* John J. Moslehi,1* and André A. Heuser1,4,†

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Metal Oxide MIE’s and AOPs in Lung Toxicity

**Soluble Metal oxides**
- ZnO
- CuO

**Transition MOX’s**
- CoO
- Co3O4
- Cr2O3
- Mn2O3
- Ni2O3

**Rare Earth Oxides**
- CeO2, Eu2O3
- Gd2O3, HfO2,
- La2O3, Sb2O3,
- Sb2O3, SnO2,
- Yb2O3, Er2O3
- Y2O3, ZrO2

**High Aspect Ratio**
- Spherical vs nanorod
  - CeO2 & TiO2

**Surface reactivity (silanols)**
- SiO2
  - fumed
  - amorphous
  - mesoporous
  - crystalline

**Redox cycling** & surface defects
- Dissolution
- Peptide
- OH-
- H2O
- H+ (pH)

**Bandgap**
- CB
- VB
- LUMO
- HOMO

**Photo activation**
- e-
- + + + ++
- + + + ++
- + + + ++
- + + + ++
- + + + ++

**Disrupt Redox equilibrium**
- Oxidative stress

**NRLP3 inflammasome**
- Sub-acute inflammation
- Membrane lysis (acute)

**NRLP3 inflammasome**
- Sub-acute inflammation
- Membrane lysis (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

Tier 1 OS
- Antioxidant defense
- Phase II enzyme
- Cytokine
- Chemokine

Tier 2 OS
- Cytotoxicity

Tier 3 OS
- Tier 2 OS
- AP-1/NFκB
- Tier 1 OS
- ROS

Mitochondrion
- Membrane lysis or perturbation
- Sub-chronic inflammation
- Autophagy

Lysosome
- NRLP3

Cell death
- Acute inflammation

Test method prioritization
- Separating key events
- Separate pathways/different doses
- Converging key events
- Test methods prioritization

Chemical
- MIE1
- KE
- MIE2
- KE
- MIE3
- KE
- AO1
- AO2
- AO3
Predictive Toxicology Approaches allows Large Numbers of Materials to be Profiled for Inhalation Tox Decisions

Transition MOx’s
- CoO
- Co₃O₄
- Cr₂O₃
- Mn₂O₃
- Ni₂O₃
- etc

High and Low Temp Silicas
- NbO
- ZrO
- Siloxane rings

Graphene
- 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

Lipid BL

NLRP3

Phosphate complexation

Rare Earth Oxides
- CeO₂
- Gd₂O₃
- La₂O
- Sb₂O₃
- Yb₂O₃
- Y₂O₃

SWCNT & MWCNT

LAR Metals/MOx

George et 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

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

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

(2015)
Will History Repeat Itself for Graphene?

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

*Nature Nanotechnology, October, 2014*
Graphene SAR Analysis as the basis for ATS, AOPs and Predictive Toxicological Paradigms

Planar surface hydrophobicity

Hydrophilic edge effects, Flake sizes

Membrane damaging AOPs (for ATS)

Surface functionalities (COOH, Epoxy, OH)

Carbonyl radicals

Membrane lysis
Lipid peroxidation
Membrane destructive lipid extraction

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

Wang et al ACS Nano 2015
Hersam Lab North-Western

Inflammasome
IL-1β (CNT Tier 1)
 UC CEIN - A Multidisciplinary Workforce

Chemistry/
Material Science

Law/Policy

Public Health

MSSR/
High Throughput

Social
Science

Ecology/
Environmental Science

Engineering

Biology/Tox/Medicine

Computational Modeling

et. al